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AIGAR NIGLAS

Effects of environmental factors on gas exchange in deciduous trees: focus on photosynthetic water-use efficiency

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Department of Botany, Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, Estonia

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications which are referred to in the text by Roman numerals:

I Sellin A, Eensalu E, **Niglas A**. 2010. Is distribution of hydraulic constraints within tree crowns reflected in photosynthetic water-use efficiency? An example of *Betula pendula*. *Ecological Research* **25**: 173–183.

II Sellin A, Tullus A, **Niglas A**, Õunapuu E, Karusion A, Lõhmus K. 2013. Humidity-driven changes in growth rate, photosynthetic capacity, hydraulic properties and other functional traits in silver birch (*Betula pendula*). *Ecological Research* **28**: 523–535.

III **Niglas A**, Kupper P, Tullus A, Sellin A. 2014. Responses of sap flow, leaf gas exchange and growth of hybrid aspen to elevated atmospheric humidity under field conditions. *AoB Plants* **6**: plu021.

IV Sellin A, **Niglas A**, Õunapuu-Pikas E, Kupper P. 2014. Rapid and long-term effects of water deficit on gas exchange and hydraulic conductance of silver birch trees grown under varying atmospheric humidity. *BMC Plant Biology* **14**: 72.

V **Niglas A**, Alber M, Suur K, Jasińska KA, Kupper P, Sellin A. 2015. Does increased air humidity affect stomatal morphology and functioning in hybrid aspen? *Botany* **93**: 243–250.

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The participation of the author in preparing the listed publications is as follows:

	I	**II**	**III**	**IV**	**V**
Original idea			*		*
Study design		*	*		*
Data collection	*	*	*	*	*
Data analysis	*	*	*	*	*
Manuscript preparation			*	*	*

LIST OF ABBREVIATION

ABA	abscisic acid
AED	atmospheric evaporative demand
A_{max}	light-saturated net photosynthesis (μmol CO_2 m^{-2} s^{-1})
A_N	net-photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
c_a	concentration of CO_2 in the atmosphere (vpm)
c_i	concentrations of CO_2 in the intercellular space of leaf (vpm)
CO_2	carbon dioxide
E	rate of transpiration (mmol H_2O m^{-2} s^{-1})
FAHM	Free Air Humidity Manipulation experiment
g_L	leaf conductance to water vapour (mol H_2O m^{-2} s^{-1})
g_S	stomatal conductance to water vapour (mol H_2O m^{-2} s^{-1})
J_{max}	maximum rate of electron transport (μmol CO_2 m^{-2} s^{-1})
K_L	leaf hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1})
$PWUE_{in}$	intrinsic water-use efficiency (μmol CO_2 mol^{-1} H_2O)
$PWUE_{inst}$	instantaneous water-use efficiency (μmol CO_2 mol^{-1} H_2O)
Q	photosynthetic photon flux density (μmol photons m^{-2} s^{-1})
RH	air relative humidity (%)
$V_{c\,max}$	the rate of Rubisco carboxylation (μmol CO_2 m^{-2} s^{-1})
VPD	atmospheric vapour pressure deficit (kPa)
VPD_L	leaf-to-air vapour pressure difference (kPa)
WUE	water-use efficiency
Ψ_B	branch water potential (MPa)
Ψ_L	leaf water potential (MPa)
Ψ_S	soil water potential (kPa)

1. INTRODUCTION

1.1. Definition of plant water-use efficiency (WUE)

Photosynthesis, a physiological process converting light energy into chemical energy, evolved in the aquatic environment. At first, photosynthesising plants were poikilohydric, depending directly on the surrounding water environment. For wide dispersal on land, plants had to keep photosynthesising cells in a well-hydrated condition in a very dry air environment compared to water. For that purpose, different structures restricting transpirational water losses evolved on the outer surface of the plant body. Due to these protective layers – dermal tissues and highly hydrophobic cuticle – plants could minimize their dependency on the surrounding environment (called homoiohydric plants) and maintain a favourable aqueous environment for photosynthesis inside the leaf cells. In addition to a favourable internal environment, photosynthesising cells need CO_2 to function. During photosynthesis CO_2 is converted into carbohydrates, which are used for growth, repairing, protecting and maintenance of tissues in plants and therefore high concentration of CO_2 ($[CO_2]$), a substrate for photosynthesis, in chloroplasts is indispensable for plants. Protective layers that lower transpiration are hardly penetrable to both water vapour and CO_2. In order to let CO_2 penetrate inside chloroplasts, tiny apertures – stomatal pores – in plant epidermis evolved. To regulate the proportions of these bidirectional fluxes (i.e. water vapour versus CO_2), plants evolved an ability to regulate stomatal pore size by adjusting the turgor of the guard cells of stomata. The ability to regulate stomatal openness and therefore stomatal conductance to water vapour and CO_2 is crucial for plants during fast changes in environmental conditions. How effectively the plant acquires carbon per lost water is expressed as water-use efficiency (WUE).

WUE refers to carbon gained per unit water lost, and can be expressed at a stand, plant or leaf level. WUE can be estimated at a certain point in time or may be integrated over time. Because of the application of different time scales and uses of the term 'water-use efficiency', this concept should be defined accurately in each particular study (Tambussi *et al.*, 2007). Agronomists and crop physiologists define WUE as accumulated plant dry matter divided by the water used for production of this amount of dry matter. This is an integrative approach consisting of two components: a temporal and spatial dimension, as dry matter accumulation takes place over long time (days, weeks, months) and accumulated dry matter includes different organs (leaves, stems and roots) (Tambussi *et al.*, 2007). Plant total biomass and the biomass of economically important parts of the plant may not be correlated; this depends on the allocation of resources within the plant. Therefore, for farmers and plant breeders, WUE of yield (biomass of economically important parts of plants divided with water used by plants) is more informative to use.

Photosynthetic water-use efficiency (PWUE) describes leaf-level water use at a certain time. PWUE measurements are carried out by applying gasometric methods at the leaf level. Two different definitions of PWUE are in usage: instantaneous photosynthetic water-use efficiency ($PWUE_{inst}$) and intrinsic photosynthetic water-use efficiency ($PWUE_{in}$). In the calculation of both, net-photosynthetic rate (A_N) is used as the numerator but different denominators characterising water consumption are used. $PWUE_{inst}$ is calculated according to Feng (1999):

$$\mathrm{PWUE_{inst}} = \frac{A_N}{E} = \frac{c_a\left(1-\frac{c_i}{c_a}\right)}{1.6\,v} \tag{1}$$

where E is the rate of transpiration, c_i and c_a are the concentrations of CO_2 in the intercellular space of leaves and in the atmosphere, respectively, and

$$v = \frac{e_i - e_a}{P} \tag{2}$$

where e_i and e_a are the water vapour pressures inside the leaf and in the ambient air, respectively, and P is the total atmospheric pressure. As seen from equations (1) and (2), $PWUE_{intr}$ depends on the vapour pressure difference (driving force of transpiration), and this dependence does not allow direct comparisons among plants growing in different environmental conditions. To overcome this obstacle $PWUE_{in}$ is used (Feng, 1999):

$$\mathrm{PWUE_{in}} = \frac{A_N}{g_S} = \frac{c_a\left(1-\frac{c_i}{c_a}\right)}{1.6} \tag{3}$$

where g_S is the stomatal conductance to water vapour. $PWUE_{in}$ is not influenced directly by water vapour pressure deficit (VPD) and thus allows comparison of plant intrinsic physiological traits among different individuals and species, thereby avoiding the effects of environmental conditions that affect the value of VPD (Feng, 1999).

$PWUE_{in}$ can be also estimated based on the fact that plants discriminate ^{13}C isotope against ^{12}C in photosynthesis and diffusion (Seibt *et al.*, 2008). Isotopic composition is specified as $\delta^{13}C$ (‰) (Farquhar *et al.*, 1982):

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000 \tag{4}$$

where R_{sample} and $R_{standard}$ are the abundance ratios ($^{13}C/^{12}C$) of the sample and standard, respectively. $R_{standard}$ is $^{13}C/^{12}C$ ratio in a Cretaceous marine fossil, *Belemnitella americana*, which is used to standardize samples. To distinguish variations in the $\delta^{13}C$ of source CO_2 from the effects of plant metabolic

processes, the $\delta^{13}C$ signature of plant organic material ($\delta^{13}C_{sample}$) is translated to photosynthetic carbon isotope discrimination (Δ; Seibt *et al.*, 2008) and is calculated according to Farquhar *et al.* (1982):

$$\Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{sample}}{1 + \frac{\delta^{13}C_{sample}}{1000}} \quad (5)$$

where $\delta^{13}C_{air}$ and $\delta^{13}C_{sample}$ are $\delta^{13}C$ values in surrounding air and in the sample, respectively. Δ and the ratio c_i/c_a are linearly related according to the model by Farquhar *et al.* (1982):

$$\Delta = a + (b - a)\frac{c_i}{c_a} \quad (6)$$

where a is the fractionation during CO_2 diffusion through the stomata (4.4‰), and b is the fractionation associated with reactions by Rubisco and PEP carboxylase (27‰). Therefore, by applying equations (4), (5) and (6) to determine the value of c_i/c_a and inserting this value into equation (3), it is possible to assess $PWUE_{in}$ indirectly, without performing gasometric measurements. This method can be used to assess $PWUE_{in}$ for short or extended periods, depending on the type of organic matter being sampled (sugars, starch, cellulose or stem material; Brueck, 2008). $PWUE_{in}$ estimated as isotopic discrimination is always integrated over shorter or longer time, whereas that estimated by gas exchange represents $PWUE_{in}$ at a particular moment. In this doctoral thesis, when $PWUE_{in}$ calculated from C isotope ratio measurements is mentioned, it is referred to as long-term $PWUE_{in}$.

1.2. Impact of environmental factors on WUE

As explained above, $PWUE_{in}$ is a quotient of net photosynthetic rate and stomatal conductance. It means that $PWUE_{in}$ depends on both A_N and g_S, which vary with environmental conditions. It is a well-known fact that plants need light for photosynthesis as light energy is used to split water molecules to generate reducing power and ATP for the production of carbohydrates. The photosynthetic light-response curves (A_N/Q curves) of different plants indicate that A_N rate grows with increasing light intensity (Larcher, 1995; González-Rodríguez *et al.*, 2002). However, the increasing irradiance does not increase A_N infinitely. At low light, photosynthesis increases linearly with increasing irradiance; when light intensity increases further, the relationship between net photosynthesis and light becomes non-linear and eventually photosynthesis becomes light-saturated with no further increases occurring with increasing light intensity. According to the model by Farquhar *et al.* (1980) photosynthesis under high light is constrained by Rubisco carboxylation rate. If leaf nitrogen

concentration increases, the nitrogen content bound in Rubisco carboxylase of leaf increases as well, which brings about higher light-saturated photosynthetic rate (Evans, 1989). It has been shown that by increasing leaf nitrogen concentration via fertilization with N, it is possible to enhance WUE due to rising A_N (Livingston *et al.*, 1999; Ripullone *et al.*, 2004; Wang *et al.*, 2010).

Another important resource for photosynthesis is CO_2 and similarly to light, rising $[CO_2]$ in carboxylation site stimulates A_N. Also similarly to the light response, A_N does not increase infinitely with rising $[CO_2]$. According to Farquhar *et al.* (1980) A_N is constrained by three constraints when $[CO_2]$ increases. As $[CO_2]$ in intercellular space (c_i) increases from its minimum concentration, dA_N/dc_i is high and determined by Rubisco activity. With further increases, there is an inflection to a lower dA_N/dc_i that approaches zero when ribulose-1,5-bisphosphate (RuBP) regeneration is limiting. In some instances, a further increase in c_i may result in another transition to a plateau or a decrease in A_N with an additional increase in c_i if triose phosphate utilization (TPU) becomes limiting (Long *et al.*, 2004).

The response of photosynthesis to temperature is parabolic in shape and decreases as temperature becomes lower or higher than the optimum (Pimentel *et al.*, 2007; Avola *et al.*, 2008) via inactivation of photochemical processes. $PWUE_{in}$ can be changed by altering chemical reactions of photosynthesis, but it changes also because of alterations in g_S. For example, an increase in atmospheric VPD reduces leaf water status via intensifying the transpiration and causing stomata to respond by reducing their apertures, restricting water loss and slowing down a further decline in leaf water potential (Ψ_L). Similar patterns in g_S regulation have been observed in relation to other hydraulically mediated environmental variables (Buckley, 2005). For instance, g_S in hybrid grapevine (*Vitis berlandieri* × *Vitis rupestris*) reduced substantially after irrigation was stopped (Pou *et al.*, 2008). When soil water content declines, abscisic acid (ABA) is synthesised in roots and transported into guard cells (Assmann and Shimazaki, 1999), or ABA is synthesised in guard cells in response to increasing VPD (Bauer *et al.*, 2013). In guard cells ABA binds with ABA receptors and this triggers a cascade of events, which leads to stomatal closure (Kim *et al.*, 2010). In addition to chemical signalling, there exists an alternative mechanism known as hydraulic signalling. Stomata close when guard cells sense a decrease in soil moisture content or increasing VPD through hydraulic signals, perceived primarily by changes in Ψ_L (Assmann *et al.*, 2000). Evidently both types of signals participate in stomatal closure in response to decreasing water content (Comstock, 2002). Regardless of the types of stomata closing signals, they do not close only as a consequence of low water availability in soil or large water losses from foliage. They close also in response to various signals, including anoxic conditions in the soil due to flooding (Jackson, 2002; Copolovici and Niinemets, 2010).

Stomata close in response to rapid decreases in air relative humidity (RH); however, on a longer time scale, increased RH also has a significant effect on

stomata. Leaves adapted to high RH have altered stomatal density and size (Torre *et al.*, 2003; Rezaei Nejad and Van Meeteren, 2005). In addition to the impact on morphology, RH also affects stomatal functioning – stomatal responsiveness. Stomata in high-RH grown plants are commonly less sensitive to the factors inducing their closure (decreasing Ψ_S, increasing leaf [ABA], darkness), demonstrating malfunctioning (Rezaei Nejad and Van Meeteren, 2005; Fanourakis *et al.*, 2013; Aliniaeifard *et al.*, 2014). Stomata in *Rosa hybrida* Vill. plants grown in high RH remain more open during desiccation, resulting in higher transpiration and more dehydrated leaves (Giday *et al.*, 2014). Stomata remain widely open in conditions where stomatal closure is beneficial, and that brings about lower leaf WUE, which may prove critical under rapidly changing environmental conditions.

At the leaf level, fluxes of CO_2 and H_2O share a part of the diffusion pathway: they both transit stomatal pores. This means that g_S is influenced by environmental factors directly (VPD, soil water potential) and indirectly via changes in the CO_2 consumption rate by photosynthesis. It has been shown that stomatal conductance may restrict A_N (González-Rodríguez *et al.*, 2002; Li *et al.*, 2010; Zhou *et al.*, 2010) and therefore A_N and g_S are coupled. The correlation between A_N and g_S depends mostly on the environment in which the leaf is located (Reinhardt and Smith, 2008) and therefore the environmental variables that alter A_N may also change g_S. Thus, the changes in PWUE may be driven by alterations in both components and when A_N and g_S (or E) change evenly in the same direction, PWUE does not change at all. Possen *et al.* (2011) demonstrated that after 37 days of application of different watering regimes, A_N and E in both silver birch (*Betula pendula* Roth.) and European aspen (*Populus tremula* L.) were lower in "dry" compared to well-watered treatment, whereas $PWUE_{inst}$ was affected by soil moisture content only in aspen trees.

Because A_N and g_S are interrelated, fluctuating environmental factors influence them both. However, the factors do not change individually *in natura*; we have to consider their combined effects on plant functioning. Interaction between high temperature and water deficit has a greater effect on photosynthesis in *Populus euphratica* Oliv. than each individual stress factor separately (Zhou *et al.*, 2010). Because of the additive effect of heat and water deficit on A_N, $PWUE_{inst}$ decreases, whereas the decrease is bigger than if the stress factors had changed separately. Both increased leaf-to-air vapour pressure difference (VPD_L) and warmer air reduce g_S; a combination of these factors results in lower stomatal conductance and respectively higher $PWUE_{in}$ (Barbour *et al.*, 2011). Soil salinity has a negative impact on A_N and g_S: they both decrease with increasing salinity (Brugnoli and Lauteri, 1991; Jiang *et al.*, 2006). Increasing [CO_2] stimulates A_N and reduces g_S, thereby enhancing $PWUE_{in}$. It has been shown that elevated [CO_2] reduces the impact of salinity on photosynthetic activity and stomatal conductance (Dehshiri *et al.*, 2015). This suggests that increasing [CO_2] is able to ameliorate the negative effects of salinity on photosynthesis and stomatal conductance, and increase WUE of productivity

(Dehshiri *et al.*, 2015). Rising [CO_2] improves plant water status also via decreasing g_S during reduced water supply (Poorter and Pérez-Soba, 2001) and therefore rising [CO_2] alleviates the negative effects of drought on plant growth rate. At the same time, the positive effect of rising [CO_2] on photosynthesis and plant WUE may be decreased when increasing [CO_2] is accompanied by a temperature rise above the optimum for plant growth (Kaminski *et al.*, 2014). Therefore the positive response of plants to single environmental variables may be restrained by other factors or multifactorial stresses.

1.3. Implication of WUE in plant acclimation in variable environment

Different strategies have developed in plant species to cope with low water availability: drought escape, drought avoidance and drought tolerance (Stebbins, 1952). Drought escape occurs when plants develop rapidly and reproduce before drought conditions become severe, i.e. they escape drought-induced damage by rapidly completing their full life cycles (Wood, 2005; Kooyers, 2015). In case of drought avoidance plants avoid the impact of drought by increasing water-use efficiency (WUE) by reducing transpiration through morphological adjustment or physiological mechanisms, limiting vegetative growth, or intensifying root growth during transient periods of drought stress (Kooyers, 2015). Drought avoiding strategy is demonstrated in trembling aspen (*Populus tremuloides* Michx) under varying atmospheric demand (Mackay *et al.*, 2003). Aspen shows high canopy transpiration under low VPD, but the transpiration rate decreases rapidly as VPD increases. Drought tolerant plant species are able to withstand suboptimal water availability by utilizing adaptations that permit metabolism to occur at low water potentials. These plants have evolved a number of anatomical, developmental, biochemical, physiological and molecular adaptations to limit the desiccation of vegetative tissues (Wood, 2005).

Many works concerning plant responses to water availability have been made with xeric plant species growing at low water availability. Xeric plants have adapted to live in arid habitats where water is scarce and therefore they generally exhibit high water-use efficiency. Also plants with C_4 carbon fixation have on average higher WUE than C_3 plants (Larcher, 1995). More efficient water use may be responsible why the frequency of C_4 plants in degraded sites, sites with disturbed water balance, is higher compared to frequency of occurrence of species with C_3 metabolism (Yu *et al.*, 2005). Therefore plants that are adapted to grow in droughty or unstable environments are able to avoid the negative effects of drought on survival by being water-use efficient. These examples refer to increased fitness of plants with higher WUE in low-water conditions. However, there is no correlation between the number of flowering stalks (measure of fitness) and long-term $PWUE_{in}$ in a natural population of herbaceus desert perennial *Cryptantha flava* (A. Nelson) Payson (Casper *et al.*,

2005), and high WUE has no adaptive significance in seedlings of western red-cedar (*Thuja plicata* Donn ex D. Don) from regions with different precipitation amounts (Fan *et al.*, 2008). Darychuk *et al.* (2012) showed that Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees from various locations with high survival are less water-use efficient (long-term $PWUE_{in}$). By contrast, Fernández *et al.* (2006) observed the highest growth rates in populations of maritime pine (*Pinus pinaster* Ait.) with the highest long-term $PWUE_{in}$. To a large extent, these conflicting results reflect differences in patterns of water availability (Nicotra and Davidson, 2010). Drought escaping is an optimal strategy in environments with short growing seasons that are followed by severe drought, whereas drought avoidance and possession of high WUE may be more suitable if the growing season is punctuated by transient mild or moderate droughts. When mild or moderate drought occurs during the whole growing season, drought tolerance strategy may be more advantageous (Kooyers, 2015).

The potential advantage for plants having higher WUE is not associated only with drought and limited soil water availability. Large plants such as trees adjust WUE even if there are no apparent drought conditions. The whole-plant hydraulic conductance decreases with increasing tree height (Martinez-Vilalta *et al.*, 2007) and according to the hydraulic limitation hypothesis (Ryan and Yoder, 1997), tree growth in height causes higher water stress in upper leaves and this triggers stomatal closure. Therefore, upper-canopy needles of *Pinus canariensis* Chr. Sm. Ex DC have higher $PWUE_{in}$ compared to lower-crown foliage (Peters *et al.*, 2008). Also Kitahashi *et al.* (2008) showed on two emergent tropical tree species that upper-canopy leaves have higher $PWUE_{in}$ compared to the lower canopy when measured under full sunlight. The ability of plants to withstand drought by adjusting WUE may change as plants grow higher and get older (Cavender-Bares and Bazzaz, 2000). Hence plants ontogeny must also be considered when assessing their acclimation and WUE in certain environmental conditions. Unfortunately, environmental conditions are seldom constant during plants' lifetime.

Plants as sessile organisms cannot move to more suitable places when environment changes unfavourably over time. They have to cope with changes in diverse environmental factors and variations at different time scales. For example, forest understory plants have to manage with quickly changing patterns of sunflecks; forest upper-canopy foliage and grassland species have to manage with cloud shadows and with co-occurring organisms that affect their light regime. Light conditions change also periodically – circadianly and annually, in addition to the dependence on leaf position in the tree canopy (Gomes-Laranjo *et al.*, 2008). Forest light conditions may alter for years after cutting or dieback of trees, thus potentially driving changes in WUE. There are circadian and annual changes in $[CO_2]$ within plant canopies and also the long-term global rising trend of atmospheric $[CO_2]$, well-documented during the 20th century and predicted to continue in the 21st century (IPCC, 2013). Also VPD and air temperature change circadianly and vary annually. Agricultural and

natural aerosols have been shown to cause an increase in leaf transpiration and a decrease in $PWUE_{inst}$ (Burkhardt *et al.*, 2001); this effect may become stronger as leaves age (Burkhardt and Hunsche, 2013).

The Intergovernmental Panel on Climate Change (IPCC, 2013) predicts an increase in average temperature and increasing frequency of extreme temperatures and droughts for the 21th century. During drought periods, plants have to cope with multiple simultaneous environmental changes: lower soil water content, higher VPD and air temperature. At higher latitudes in northern Europe, increased precipitation in addition to rising surface temperature are also predicted during the 21st century (IPCC, 2013). Increasing rainfall frequency with rising temperature means higher relative humidity at local or regional scales. Plants have to acclimate to different environmental factors but in nature these factors rarely change alone, thus the plants have to respond simultaneously to multiple changes and this response differs from that of separate environmental variables.

Plant water-use efficiency depends not only on water availability, but on accessibility to all main environmental resources, including light. The capability of plants to adjust their WUE quickly in variable environments is of great ecophysiological importance. There is a range of adaptations developed during evolution allowing plants to live in specific light conditions. Species from bright light environments respire at higher rates under low photosynthetic photon fux densities (Q) and require more light to achieve a zero net carbon uptake and to saturate photosynthesis while achieving higher rates of photosynthesis at high irradiance than species found in shady habitats (Givnish *et al.*, 2004). Species adapted to live in sunny habitats are generally characterised by higher photosynthetic rates per unit leaf area and more open stomata compared to those inhabiting shaded areas (Givnish, 1988; Kubiske *et al.*, 1996). When g_S does not change, increasing A_N brings higher $PWUE_{in}$. At the same time, increased A_N requires higher g_S to reduce stomatal limitation on photosynthesis. Higher Q means also higher leaf temperature, which may also impel an increase in stomatal conductance to prevent A_N loss via temperature damage on photosynthetic apparatus. Therefore higher A_N in high-light adapted plants or acclimated leaves does not mean automatically higher $PWUE_{in}$ when g_S is also higher. This is illustrated by *Cypripedium flavum* P.F.Hunt et Summerh. plants grown in well-illuminated conditions versus shady environment. Plants acclimated to high light levels have higher A_N in saturated light conditions because of raised nitrogen-use efficiency and greater mesophyll conductance of CO_2. At the same time $PWUE_{in}$ did not differ between the light conditions as A_N and g_S rose equally (Li *et al.*, 2008).

The advantage having higher WUE in changing environment depends on species' adaptations to certain environments. Species shade tolerance and drought tolerance are inversely related (Niinemets and Valladares, 2006), meaning that shade tolerant, usually understory plants, are less drought tolerant. When light conditions improve, $PWUE_{in}$ of shade tolerant *Acer* species rises

(Hanba *et al.*, 2002), which is associated with improved drought avoiding strategy, especially under mild drought. As explained hereinabove, a mild water deficit may occur in trees even if there is plenty of water in soil. Shade-tolerant *Acer saccharum* Marsh. shows little change in physiological characteristics after cap formation in a forest stand. Light-saturated photosynthesis (A_{max}) did not increase after shade leaves were exposed to brighter light conditions, but g_S increased after one year (Jones and Thomas, 2007). Lavinsky *et al.* (2007) demonstrate that plants acclimated to different light levels adjust $PWUE_{in}$ differently when they encounter flooding. Also, $PWUE_{inst}$ of sun and shade-adapted leaves respond differently to increasing CO_2 levels (Kubiske and Pregitzer, 1997) or to different seasons (Peters *et al.*, 2008). Hence, light acclimation plays an important role in plants' ability to adjust their water use and survival under changing environment.

Plants growing in the Mediterranean region characterized by frequent droughts usually have high WUE, which can also be modified by other environmental variables. Luis *et al.* (2010) demonstrated that seedlings of *Pinus canariensis* grown at higher nitrogen levels have higher A_N, but there are no differences in transpiration rate or g_S between the seedlings acclimated to different fertilization levels. Thus, higher PWUE is an outcome of acclimation of A_N but not g_S to different nutrient availabilities, and increased N availability helps to overcome water deficit. Before increasing N deposition, the variation in long-term $PWUE_{in}$ in *Quercus cerris* L. stands was driven mainly by climate via regulating g_S (Guerrieri *et al.*, 2010). After the increase in N-compounds in the atmosphere, the relative role of g_S in long-term $PWUE_{in}$ remained the same irrespective of N availability; however, higher N availability represented the key factor that caused an increase in A_N and $PWUE_{in}$ in high N sites. Consequently, increased N deposition may improve the balance between carbon assimilation and water loss, especially under the scenario of significant reduction of precipitation predicted for Mediterranean area (Guerrieri *et al.*, 2010).

The atmospheric [CO_2] concentration has steadily increased since the second half of the 19th century. Numerous lab experiments have proved the positive effects of elevated [CO_2] on A_N and plant WUE in the short term (Teramura *et al.*, 1990; Avola *et al.*, 2008; Kaminski *et al.*, 2014). Is this effect observable also on a long time scale? Long-term $PWUE_{in}$ of Qilian juniper (*Sabina przewalskii* Kom.) and Qinghai spruce (*Picea crassifolia* Kom.) growing in arid ecosystems has increased over the 150-year period, caused mainly by increasing atmospheric [CO_2] (Liu *et al.*, 2007). The magnitude of plant water-use response to rising [CO_2] varies among biomes (Tian *et al.*, 2010), depending on species adaptation to specific environmental conditions (Liu *et al.*, 2007). At the same time tree increments have not increased with rising long-term $PWUE_{in}$ as was expected (Silva *et al.*, 2010; Peñuelas *et al.*, 2011; Silva and Anand, 2013). The impact of atmospheric changes, primarily an increase in [CO_2], on forest productivity depends on latitude (Zhu *et al.*, 2011; Silva and Anand, 2013). The

potential growth benefits from growing in a higher [CO_2] world may be overridden by other factors such as nutrient limitation and/or long-term physiological acclimation to elevated [CO_2] or concurrent climate variables, particularly drought (Peñuelas *et al.*, 2011; Silva and Anand, 2013). Linares *et al.* (2009) report that both high- and low-elevation populations of *Abies pinsapo* Boiss. show an increase in long-term $PWUE_{in}$ with rising atmospheric [CO_2] from 1950 to 2005. However, the rate of the increase in long-term $PWUE_{in}$ at lower elevations (dryer sites) appears to be insufficient to counteract the growth decline induced by warming and more xeric conditions. Therefore, the rise in long-term $PWUE_{in}$ is probably not caused merely by rising [CO_2] over a long time, but co-occurring factors (water deficit) affecting stomatal responses may also be involved.

The examples hereinabove suggest that high WUE is not always beneficial to plants and depends on species' adaptations and given environmental conditions. High WUE could be beneficial in certain conditions by enhancing plant fitness, but not necessarily in others. Plants with lower long-term $PWUE_{in}$ have significantly higher growth rate in wet years, but not in dry years (Ramirez-Valiente *et al.*, 2010). Taylor *et al*. (2011) show that C_4 plants have a photosynthetic advantage (and therefore high $PWUE_{in}$) over C_3 plants in mesic conditions, but this advantage is lost under drought conditions. Nicotra and Davidson (2010) compare "water-wise" plants with a "water-wise" dishwasher: plants should conserve water only when water is limiting, unlike a "water-wise" dishwasher, which always conserves water. Therefore, high plasticity in WUE in fast-changing environments is evidently more beneficial than a statically high WUE. Nicotra and Davidson (2010) observed significantly higher plasticity in WUE in invasive plants compared to native species, thereby explaining their success in new environments. Furthermore, conifer species with more variable $PWUE_{in}$ from early to late summer are considered less vulnerable to environmental changes (Grulke, 2010). An interesting plastic adaptation of photosynthesis to drought occurs in inducible CAM (crassulacean acid metabolism) plants (Chaves *et al.*, 2003). Inducible CAM plants generally operate under C_3 mode of photosynthesis, but once exposed to dehydration stress, they switch to more water-use efficient CAM photosynthesis (Chaves *et al.*, 2003), attesting to the beneficial effect of high WUE only in conditions of low water availability. Pioneer species that inhabit open habitats characterized by high variability of environmental conditions (irradiance, water availability, soil and air temperatures) have usually higher $PWUE_{in}$ compared to late-sucessional forest species adapted to live in relatively stable environment (Nogueira *et al.*, 2004). In *Arabidopsis thaliana* (L.) Heynh. the long-term $PWUE_{in}$ and plasticity in long-term $PWUE_{in}$ are positively correlated (Kenney *et al.*, 2014).

1.4. Aims of the thesis

According to the hydraulic limitation hypothesis (Ryan and Yoder, 1997), hydraulic resistance increases as the tree grows taller and begins to constrain water supply to distal foliage. Thus, the growing length of the water transport pathway may induce water stress in leaves leading to stomatal closure. Studies in forest stands with trees in natural light conditions have demonstrated higher PWUE in leaves growing in upper canopy layers compared to lower-canopy leaves (González-Rodríguez *et al.*, 2002; Peters *et al.*, 2008). Sellin and Kupper (2005) found that the mean hydraulic conductance of the soil-to-leaf transport pathway is 1.7–1.8 times higher for the upper canopy compared to the lower canopy in 16–17 m *Betula pendula* Roth. trees growing in a natural stand. At first glance, this seems to contradict the hydraulic limitation hypothesis, as stomata in lower leaves are potentially more limited hydraulically and therefore we suggest that lower leaves should have higher PWUE for normal functioning.

Acclimation to different environmental factors affects competition capability and the survival of the plant in stress conditions, including periods of low water availability. One of the factors is atmospheric humidity; however, there is a lack of knowledge on how acclimation to certain RH levels affects trees' gas exchange and growth *in natura*, in diurnally and seasonally variable environments. The study is focussed on the impact of rising air humidity due to increasing rainfall frequency – a climate trend predicted for northern latitudes – on gas exchange in trees. The consequences of this climate trend on forest ecosystem functioning is still a greatly understudied aspect of climate change. Therefore, the present thesis aims to understand how growing in increased RH affects stomatal responses in deciduous trees and how the trees adjust their gas exchange if leaves encounter a long-term and rapid decrease in water supply. In addition to the response to water stress (limited water supply), we try to clarify how stomata respond to fast increase in air vapour pressure deficit in leaves developed in elevated RH. Most of the studies were carried out in field conditions – in naturally established forest stands or in an experimental forest plantation.

This thesis aimed at the following objectives:

- To study the vertical and temporal variation in leaf gas exchange, with focus on photosynthetic water-use efficiency, in a deciduous tree species within a natural forest canopy (**I**).
- To assess the possible effects of artificially elevated relative air humidity on basic leaf gas exchange parameters of broadleaved trees (**II**, **III**).
- To examine how gas exchange of trees grown under different levels of atmospheric humidity respond to rapid and long-term water deficits (**IV**).
- To ascertain the stomatal response to rapid increases in atmospheric vapour pressure deficit in leaves developed in elevated RH compared to those in ambient conditions (**V**).

The key hypotheses set up and tested in the thesis:

1. As lower-canopy leaves growing in shade conditions are hydraulically more constrained compared to upper foliage, shade leaves have intrinsically higher water-use efficiency compared to sun leaves.
2. Trees growing under increased relative atmospheric humidity develop leaves with lower photosynthetic water-use efficiency.
3. Stomata of trees grown in higher atmospheric humidity are less sensitive to stomatal closure inducing factors (water deficit, increasing atmospheric vapour pressure deficit).

2. MATERIALS AND METHODS

2.1. Experiment with large forest trees (Paper I)

Five 20–30 year old neighbouring silver birches (*Betula pendula*) accessible from an access tower were selected for measurement. The trees were growing in the transition zone of the *Oxalis* boreal and *Filipendula* paludifying forest site types, in Järvselja Experimental Forest (58°16′ N, 27°16′ E, elevation 40 m ASL) in eastern Estonia. The average annual precipitation in the region is 650 mm; the average temperature is 17.0 °C in July and –6.7 °C in January. The growing season usually lasts 175–180 days, from mid-April to October. The annual sum of the global short-wave radiation in the region averages 3518 MJ m^{-2}, and the annual radiation budget is 2552 MJ m^{-2}. The soil is a gleyed pseudopodsol formed on a loamy till and is characterized by a large water storage capacity. The study was conducted from June to August in 2008.

Sampling in the forest in situ

Leaf gas exchange parameters were measured in shade (9.5–12 m above the ground) and sun folige (15–16.5 m above the ground) accessed from the scaffolding tower installed between the trees. Gas exchange was measured on fully expanded leaves with a portable photosynthesis system LCpro+ (ADC BioScientific, Great Amwell, UK) at ambient irradience, temperature, air humidity, and at constant external CO_2 concentration (c_a=370 vpm). Measurements were taken at 2-h intervals from 07:00 to 17:00 hours, East European standard time; two to three leaves per tree and per canopy layer were sampled at each measurement time. Soil water potential (Ψ_S; MPa) at depths of 20, 40, and 60 cm was recorded with EQ2 equitensiometers (Delta-T Devices, Burwell, UK). Basic atmospheric variables (photosynthetic photon flux density, air temperature, air relative humidity, wind speed) were recorded continuously in the lower canopy layer and immediately above the canopy. All environmental data were stored with a DL2e data logger (Delta-T Devices).

Laboratory experiment

For generating photosynthetic light response curves (A_N/Q curve), birch shoots 20–30 cm in length were cut under water from upper (sun leaves) and lower thirds (shade leaves) of canopy and transported to the laboratory with the basal ends submerged in water. In the laboratory, the shoots were put into plastic flasks filled with deionized, filtered (Direct-Q3 UV water purification system; Millipore SAS, Molsheim, France) and freshly degassed water (T-04-125 ultrasonic-vacuum degasser; Terriss Consolidated Industries, Asbury Park, NJ), and were illuminated under Q of 610–630 μmol m^{-2} s^{-1} (SON-T AGRO high-pressure sodium lamps, 400 W; Philips, Eindhoven, The Netherlands) for at

least 1 h before measurements. The measurements were performed on five to eight leaves per tree and per canopy layer with a LCpro+ equipped with a LED light source, while maintaining constant temperature of the leaf chamber (25°C) and c_a (370 vpm). The measurements started at Q of 1,196 μmol m^{-2} s^{-1}, then Q was decreased stepwise to 9 μmol m^{-2} s^{-1} and increased stepwise from 1,196 to 1,601 or 1,803 μmol m^{-2} s^{-1} for shade and sun leaves, respectively.

2.2. Experiments at Free Air Humidity Manipulation site (Papers II–V)

The Free Air Humidity Manipulation (FAHM) site in an experimental forest plantation, situated at Rõka village (58°14′N, 27°17′E, 40–48 m ASL), Eastern Estonia. The FAHM site is located about 5 km away from the previous study site (natural mixed forest stand), thus has the same climatic conditions. The soil is a fertile Endogleyic Planosol (IUSS Working Group WRB, 2007) with an A-horizon thickness of 27 cm. Total nitrogen content is 0.11–0.14%, C/N ratio is 11.4, and pH is 5.7–6.3. The site represents a fenced area of 2.7 ha containing nine circular experimental plots (diameter 14 m) planted with hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) and silver birch and surrounded by a hybrid aspen buffer zone. One-year-old micropropagated hybrid aspen plants and seedlings of silver birch were planted in the experimental area in 2006. In 2012 the trees were felled and the plots replanted (silver birch) or coppice shoots (stump and root sprouts) were allowed to emerge (hybrid aspen).

The stand density in the buffer zone is 2,500 trees ha^{-1}, and in the experimental plots, 10,000 trees ha^{-1}. Three sample plots were used as control (**C**) areas and in three plots the air humidity was increased, i.e. humidified plots (**H**). The computer-operated FAHM system, based on an integrated approach of two different technologies – a misting technique to atomize/ vaporize water and FACE-like technology to mix humidified air inside the plots – enables RH of the air to increase by up to 18% over the ambient level during the humidification application, depending on the wind speed inside the experimental stand. The humidification is applied during daytime 6 days a week throughout the growing season if ambient RH is <75% and mean wind speed is <4 m s^{-1}.

In each experimental plot the main environmental parameters are routinely measured: air temperature (T_a) and RH (HMP45A humidity and temperature probe; Vaisala, Helsinki, Finland); photosynthetically active radiation (Q) above the canopy (LI-190SZ quantum sensor; LI-COR Biosciences, Lincoln, NE); soil water potential (Ψ_S) at depths of 15 and 30 cm (EQ2 equitensiometer; Delta-T Devices). Sensor readings are collected and stored as average values every 1–10 min with a DL2e data logger (Delta-T Devices). A detailed description of the FAHM site and technical setup is presented in Kupper *et al.* (2011).

Gas exchange measurements

Studies **II** and **III** were conducted from July to August, 2009 and 2011, respectively. Model plants of silver birch (six trees per treatment; **II**) or hybrid aspen (nine trees per treatment; **III**) growing at the FAHM site were chosen and sampled gasometrically. All gasometric measurements were performed on rainless misting-free days on intact healthy, fully expanded leaves in situ with a LCpro+ photosynthesis system at ambient (**II**) or constant air humidity (water vapour pressure 13 mbar; **III**) while maintaining constant temperature of the leaf chamber (25°C) and ambient CO_2 concentration (c_a=360 vpm). Vapour pressure deficit in the cuvette for **C** and **H** plants was on average 0.35 and 0.39 kPa (**II**) or 2.12 and 1.99 kPa (**III**), respectively.

To generate photosynthetic light response curves (A_N/Q curves), four leaves per tree were sampled from the middle part of the crown with the photosynthesis system equipped with an LED light source. The measurements started with Q at 1,196 μmol m^{-2} s^{-1}, then decreased stepwise to 9 μmol m^{-2} s^{-1} and increased stepwise from 1,196 to 1,803 μmol m^{-2} s^{-1}. The response of net photosynthesis to varying intercellular CO_2 concentration (c_i) – A_N/c_i curves – was also determined on intact leaves (four leaves per tree) in situ at constant air humidity (water vapour pressure 13 mbar), temperature of the leaf chamber (25°C) and at saturating irradiance (1,500 μmol m^{-2} s^{-1}). External CO_2 concentration (c_a) was supplied at 11 steps, decreasing from 360 to 60 μmol mol^{-1} and then increasing from 450 to 1,600 μmol mol^{-1}.

Study **IV** was conducted from June to July, 2010. Gas exchange measurements were performed on 15 **H** and 15 **C** silver birches growing at the FAHM site. One sample branch (mean height above the ground 140±9.3 cm for **C** trees and 138±8.4 cm for **H** trees) per tree from the middle third of the crown was selected for gasometric measurements, which were conducted on four or five leaves per branch at a saturating photosynthetic photon flux density (1,196 μmol m^{-2} s^{-1}) applying constant c_a (360 μmol mol^{-1}), air humidity (15 mbar) and temperature (25ºC) with a LCpro+. Simultaneously leaf conductance to water vapour (g_L) was measured in ambient condidions with LI-1600M steady-state porometer (LI-COR Biosciences). The first measurements were performed immediately before branch cutting. After the sample branches were cut off, they were allowed to dehydrate in open-air in order to generate a water deficit, the next measurements were conducted within ~3 h after cutting. All measurements were done on dry leaves under non-misting conditions: on intact branches in the morning before misting started and after that outside the experimental plots.

In study **V**, coppice shoots of hybrid aspen were sampled for gas exchange from July to August, 2013. One fully expanded leaf from each of 37 coppice shoots from **H** and **C** treatments was measured gasometrically with a LCpro+ equipped with an LED light source, while maintaining constant temperature (26 °C) and c_a (370 vpm) in the leaf chamber, and constant illumination (Q=1,398 μmol m^{-2} s^{-1}). The measurements were conducted at four levels of air

relative humidity (RH) inside the leaf chamber: 80% or ambient (if external RH<80%), 60%, 40% and 20%. Measurement cycles started at highest level of RH and decreased stepwise to 20%.

Leaf water potential and hydraulic conductance

Bulk leaf water potential (Ψ_L; studies **IV** and **V**) was measured by the balacing pressure technique (Boyer, 1995; Holbrook *et al.*, 1995) using a Scolander-type pressure chamber. Xylem water potential of the branches (Ψ_B; study **IV**) was assessed by applying the bagged leaves technique (Nardini *et al.*, 2001; Brodribb and Holbrook, 2003). Water potential of the non-transpiring (bagged) leaves, presumed to have equilibrated with the xylem water potential of the branch proximal to the petiole, was taken as an estimate of Ψ_B.

In study **II**, hydraulic conductance of whole branches and their parts (leafless branch, petioles, leaf blades) and specific hydraulic conductivity of wood samples was determined by the water perfusion method using a high pressure flow meter (HPFM; Dynamax, Houston, TX) applied in a quasi-steady-state mode. The data on branch and stem hydraulic characteristics were corrected for the dynamic viscosity of water at 22 °C and the hydraulic conductance was normalised to the foliage area supported by their corresponding branch or stem sections.

In study **IV**, leaf (K_L), soil-to-branch (K_{S-B}) and whole-tree hydraulic conductances (K_T) were estimated by the evaporative flux method under steady-state conditions and calculated according to the Ohm's law analogy:

$$K = \frac{E}{\Delta\Psi} \tag{6}$$

where E is the evaporative flux measured at the leaf surface and $\Delta\Psi$ is the water potential drop across the corresponding segment (Ψ_B-Ψ_L, Ψ_S-Ψ_B and Ψ_S-Ψ_L, respectively). As E is expressed per unit leaf area, values of K_L have been scaled by leaf area. K_L was standardized for the dynamic viscosity of water at 28 ºC, K_{S-B} and K_T were left unstandardized, because of variable temperature along these long transport pathways.

Tree growth rate and leaf nutrient contents

Trees growing in experimental plots at the FAHM site were assessed annually for height and stem basal diameter measured at 0.3 m above the ground. Height and radial growth rates were calculated from the height and stem basal diameter records made after the growing season in consecutive years.

In study **II**, leaf samples were collected from each treatment and analysed for total Kjeldahl nitrogen and total Kjeldahl phosphorus. Block digestion and steam distillation methods were used to measure N concentration (Tecator digestion system AN 300). Digestion by flow injection analysis (Tecator AN

5242) was used to pretreat the leaf material for P analysis by FIAstar 5000 analyser (FOSS Analytical, Hilleroed, Denmark).

2.3. Data analysis

Intrinsic water-use efficiency ($PWUE_{in}$) was calculated as the ratio of net photosynthesis (A_N) to stomatal conductance to water vapour (g_S). In papers **II** and **III** $PWUE_{in}$ was estimated at two levels of irradiance: 400–600 μmol m^{-2} s^{-1} when $PWUE_{in}$ was usually at a maximum and at light intensities corresponding to full sunlight ($Q \geq 1{,}400$ μmol m^{-2} s^{-1}). In addition, initial $PWUE_{in}$ was determined in study **III** based on initial values of the A_N/Q and A_N/c_i sequences when c_a was 360 vpm.

Data of gas exchange at different light intensities were fitted to different empirical models: Michaelis–Menten function modified for dark respiration rate (Givnish *et al.*, 2004; Equation 3 in **I**) and non-rectangular hyperbola expressed as a quadratic equation (Prioul and Chartier, 1977) in papers **II** and **III**. The A_N/c_i curves in studies **II** and **III** were analysed according to the biochemical model proposed by Farquhar *et al.* (1980) and subsequently modified by Harley and Sharkey (1991) and Harley *et al.* (1992). A_N/Q and A_N/c_i curves were analysed with Photosyn Assistant, Ver. 1.2 software (Dundee Scientific, Dundee, UK) in papers **II** and **III**, and by using Statistica, Vers. 7.1 (StatSoft Inc., Tulsa, OK) in paper **I**. Leaf-to-air vapour pressure difference (VPD_L) was estimated as the difference between saturated vapour pressure at the leaf temperature and the vapour pressure inside the leaf chamber (**I**, **V**). Saturated vapour pressure was calculated according to Buck (1981) (Equation 1 in **I**).

Routine statistical data analyses were carried out using Statistica software. To analyze the effects of categorical and continuous factors on dependent factors, analysis of variance (ANOVA) or when appropriate nested ANOVA was used. Type III or type IV (in case of unbalanced data sets) sums of squares were used in the calculations. Effect sizes were assessed by partial eta-squared ($\eta^2_{partial}$) defined as the sum of squares for a given effect divided by the sum of squares for a given effect plus the sum of squares for the respective error term. To assess whether the categorical dependent binary variables depend on continuous variables, we applied logistic regression. Normality and homogeneity of variances were checked using the Kolmogorov–Smirnov D-statistic and the Levene test, respectively. When appropriate, logarithmic or complex transformations were applied to the data. Post hoc mean comparisons were conducted using the Tukey HSD test. Bivariate relationships between the studied characteristics and independent variables were assessed by Pearson's correlations and simple linear or non-linear regressions based on the least-squares method.

3. RESULTS AND DISCUSSION

3.1. Vertical and temporal variation of leaf gas exchange within a forest canopy (I)

Mean values of net photosynthesis (A_N) in silver birch followed the course of light intensity (Q) in both canopy layers during the day. A_N rose in the morning with increasing Q, levelled off between 09:00 and 13:00 h and decreased with Q in the afternoon. There were no qualitative differences in the temporal course of A_N between the two canopy positions, but A_N was significantly lower throughout the day in the lower-canopy leaves compared to upper-canopy leaves (Fig. 1 in **I**). The daily mean A_N was 4.5±0.1 and 8.0±0.1 μmol m^{-2} s^{-1} in lower and upper-canopy leaves, respectively. This is an expected result as leaves in the lower canopy receive less light throughout the day compared to upper-canopy leaves and their photosynthetic machinery has acclimated to lower levels of irradiance. It has been shown that maximum values of A_N in *Persea indica* (L.) K. Spreng. in the lower canopy are about 25% of those in upper-canopy leaves (González-Rodríguez *et al.*, 2002). As the net photosynthetic rate is determined primarily by photosynthetically active radiation, A_N and Q were well correlated (r=0.668, P<0.001 for sun leaves and r=0.647, P<0.001 for shade leaves) in both canopy layers.

Stomatal conductance (g_S) increased in the morning, achieved the highest values around 09:00 h and then steadily declined, whereas the decline in upper-canopy leaves was steeper. The greater decline in g_S in upper canopy resulted in higher intrinsic photosynthetic water-use efficiency ($PWUE_{in}$) of sun foliage in the afternoon (Fig. 1c in **I**). $PWUE_{in}$ increased with rising Q, achieved a maximum by 13:00 h and then started to decline. This temporal pattern reflects well trees' acclimation to the daily course of atmospheric evaporative demand (AED) in natural conditions – daily maximum WUE coincides with maximum AED in the midday period. $PWUE_{in}$ did not differ between the canopy layers from 07:00 to 11:00 h. The sample trees of silver birch were of similar size, thus no size effects were observed. However, taller coast redwood (*Sequoia sempervirens* (D.Don) Endl.) trees demonstrate smaller variation in g_S throughout the day compared to shorter trees, also smaller g_S response to changing Q and VPD (Ambrose *et al.*, 2010) attesting to size-related hydraulic effects on diurnal gas exchange. As lower-canopy leaves in our sample trees were hydraulically more limited (Sellin and Kupper, 2005), the smaller decline in g_S during the day is consistent with the findings by Ambrose *et al.* (2010). Because hydraulic constraints increase with a tree height, the predawn and midday leaf water potentials (Ψ_L) tend to decline with tree height. Moreover, taller trees have larger difference between the predawn and midday Ψ_L and therefore the leaves of higher trees may suffer water stress at midday (Kenzo *et al.*, 2015). In our study Ψ_L was lower in the upper canopy throughout the day compared to shade foliage, whereas the minimum was observed after midday (at 13:00 h; Fig. 2

in **I**). Respectively, beginning from 13:00, $PWUE_{in}$ in the upper canopy exceeded that of lower-canopy leaves. The hydraulic differences existing within the crowns of silver birches (Sellin and Kupper, 2005) were offset by regulation of g_S.

Plant species differ in their capacity to adjust their WUE in response to variation in environmental factors, depending on their adaptations to certain conditions. Mediterranean species *Phillyrea latifolia* L. is able to increase its $PWUE_{inst}$ (by 56%) in sunlit leaves during midday under drought conditions by lowering its transpiration rate (Ogaya and Peñuelas, 2003), whereas tropical rain forest trees that maintain high photosynthesis with less stomatal closure are more successful under moist conditions and do not need to hold constant midday leaf water potential via regulating transpiration through sensitive stomatal control (Kenzo *et al.*, 2015). In our study, upper-canopy leaves of *B. pendula* had higher g_S and lower Ψ_L throughout the day, which means higher water stress at midday for the upper canopy, followed by greater decrease of g_S and increase of $PWUE_{in}$ even under high soil water availability.

Across the whole dataset sun leaves (upper canopy) of silver birch demonstrated higher $PWUE_{in}$ than shade leaves (lower canopy layer). This is consistent with a number of studies showing that leaves growing in full sunlight exhibit higher WUE compared to shade-grown leaves (Stokes *et al.*, 2010) and that forest-growing trees have higher WUE (estimated by both gas exchange and carbon isotopic composition) in leaves from upper canopy, from well-illuminated conditions, compared to lower canopy because of the differential effect of light intensity on A_N and g_S (Niinemets *et al.*, 1999; Helle and Schleser, 2004; Peters *et al.*, 2008). Le Roux *et al.* (2001), based on simulations on *Juglans regia* L., showed that under equal light conditions, there is no WUE gradient within the tree canopy. When the data on *B. pendula* were analyzed across definite light intensity intervals, $PWUE_{in}$ differed between the two canopy layers only under low-light conditions (Q<200 μmol m^{-2} s^{-1}). When 200≤Q<800 μmol m^{-2} s^{-1} or Q>800 μmol m^{-2} s^{-1}, the sun and shade foliage did not differ in $PWUE_{in}$ (white and black columns, respectively, in Fig. 1). Moreover, at midday (i.e. under high AED) and under similar light conditions (Q>800 μmol m^{-2} s^{-1}), $PWUE_{in}$ was significantly higher in shade leaves compared to sun leaves (28.8 μmol mol^{-1} versus 24.0 μmol mol^{-1}; P<0.01). This result indicates that lower $PWUE_{in}$ in shade leaves of silver birch is attributable primarily to low light availability limiting photosynthetic carbon assimilation.

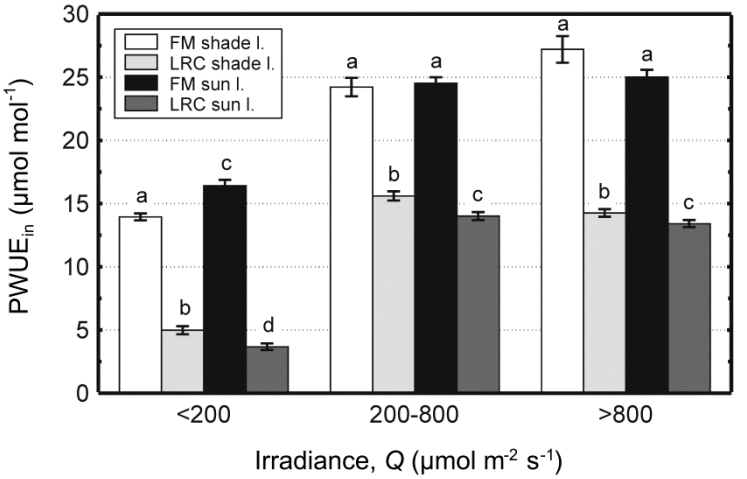

Figure 1. Comparison of intrinsic water-use efficiency ($PWUE_{in}$) in silver birch between the two canopy layers across definite intervals of irradiance. FM – field data (n=1928); LRC – data from the light response curves (n=1453). Bars indicate ±SE of the means; different letters denote statistically significant (P<0.05) differences.

An experiment conducted on forest-growing sugar maple (*Acer saccharum*) trees by Coble and Cavaleri (2015) indicates that long-term $PWUE_{in}$ increases with leaf height from the ground, but does not change with shading, suggesting that leaf height is a more influential factor than light for water-use efficiency and stomatal behaviour. In *Sequoia sempervirens*, height above the ground correlates substantially stronger to leaf anatomical traits than does light availability (Oldham *et al.*, 2010). These findings call into question the use of terms "sun" and "shade" when referring to leaves at the top or bottom of the tree crown (Oldham *et al.*, 2010). In our study the photosynthetic parameters obtained from A_N/Q curves (Table 3 in **I**) exhibit typical biochemical characteristics of leaves acclimated to sunny and shady environments (Larcher, 1995), but these leaves were hydraulically constrained differently, therefore we cannot neglect leaf acclimation to height causing differences in hydraulic conductance (Sellin and Kupper, 2005). Although we detected an impact of light conditions on photosynthetic and stomatal traits, the effect of hydraulics on gas exchange still exists.

An artificial reduction of hydraulic constraints – removal of root, stem or branch resistances – significantly influences leaf gas exchange. Reduction of hydraulic constraints increased $PWUE_{in}$, A_N and g_S in *Austrocedrus chilensis* (D.Don) Pic.Serm. et Bizzarr after branches were cut from trees and measured in the laboratory (Gyenge *et al.*, 2008). A reduction in hydraulic constraints (measured on cut shoots) in silver birch relieved stomatal control, leading to a 1.9–2.5-fold increase in g_S and a 1.2–1.7-fold increase in A_N compared to the intact foliage (Fig. 4 in **I**). Stomatal conductance in detached shoots increased

more in the shaded part of crown: at moderate to high light intensities g_S was on average 2.1–2.2 times higher compared to the intact shoots. These results confirm that g_S in forest trees is constrained by hydraulic limitations and confirm that lower-canopy leaves in *B. pendula* are hydraulically more constrained as attested in previous studies (Sellin and Kupper, 2005; Eensalu *et al.*, 2008; Sellin *et al.*, 2008).

The results obtained in *B. pendula* suggest that lower-canopy leaves of trees growing in natural forest stands have higher $PWUE_{in}$ at midday period than upper leaves under sufficient light availability. When gas exchange is measured on detached shoots under the same light conditions in the laboratory, $PWUE_{in}$ is always higher in the leaves from the lower part of the canopy (grey columns in Fig. 1). Consequently, in natural conditions – within a forest canopy – $PWUE_{in}$ is lower in lower-canopy leaves primarily because of light limitation on photosynthesis. When most of the resistance to long-distance water transport is eliminated, one can see that shade foliage is intrinsically more water-use efficient, indicating acclimation to higher soil-to-leaf hydraulic resistance. Valladares *et al.* (2008) showed that shade-tolerant *Quercus ilex* L. has higher $PWUE_{in}$ when growing in shaded compared with sunny habitats. Higher $PWUE_{in}$ enhances the performance of this species in an understory light environment characterized by numerous and intense sunflecks. In the lower canopy of *B. pendula*, high $PWUE_{in}$ reflects acclimation of leaf physiology to specific light conditions – low light intensity (Fig. 1d in **I**) combined with a highly dynamic sunfleck pattern, and to bigger hydraulic constraints. One cannot underestimate sunflecks as an important resource of light energy for plants or leaves inhabiting shady environments, but we have to consider also the potential for stress during sunflecks from excess solar radiation, temperature or VPD (Leakey *et al.*, 2005). It is a critical issue for *B. pendula*, a fast-growing pioneer species characterized by the greatest vulnerability to cavitation among a number of temperate broad-leaved tree species (Barigah *et al.*, 2006), if water loss is not controlled efficiently.

The role of plant hydraulic capacity in formation of leaf WUE has been proved by several researchers who established a strong inverse relationship between hydraulic conductivity and both long-term (Cernusak and Marshall, 2001) and short-term $PWUE_{in}$ (Taylor and Eamus, 2008; Chen *et al.*, 2009) in various woody species. Our study in *B. pendula* revealed a height-related trend in $PWUE_{in}$ opposite to that in soil-to-leaf hydraulic conductance ascertained on the same sample trees (Sellin and Kupper, 2005), referring to the trade-off between water transport and use efficiencies. This represents an internal adjustment of stomatal responses in plants in relation to water supply in order to cope in the spatially and temporally heterogeneous environments existing in forest canopies. The trade-off between plant hydraulic capacity and water-use efficiency can develop over a long period and is associated with acclimation to specific conditions. Therefore, changes in $PWUE_{in}$ induced over a short time scale (in days) are uncoupled with hydraulic conductance, although the latter

may alter as well (Sellin *et al.*, 2013). We observed a trade-off between hydraulic conductance and intrinsic WUE also in saplings of silver birch (**IV**). $PWUE_{in}$ in intact branches declined with increasing plant hydraulic capacity: with leaf (R^2=0.204, P<0.05), soil-to-branch (R^2=0.356, P<0.01) as well as whole-tree hydraulic conductance (R^2=0.356, P<0.01).

Our results do not contradict the hydraulic limitation hypothesis. According to the hypothesis, hydraulic resistance of trees decreases as trees grow taller because of the longer overall path length of water flow (Ryan and Yoder, 1997) and this may constrain g_S and lead to higher leaf WUE. Our sample trees were of similar height. Hydraulic differences in their crowns result primarily from vertical environmental gradients existing within the forest canopy and from long-term acclimation of branch and leaf structure to these conditions. As shade leaves of tall birch trees growing in natural forest stand are hydraulically more constrained, they have higher $PWUE_{in}$.

Stomatal morphology is also very likely involved in long-term adjustment of plant water-use efficiency. Different poplar (*Populus*) species have different stomatal densities that are associated with their long-term $PWUE_{in}$ (Cao *et al.*, 2012). Plants of perennial grass *Leymus chinensis* (Trin.) Tzvel. grown at different soil water contents have differences in stomatal densities that are associated with $PWUE_{inst}$: higher stomatal density brings about higher $PWUE_{inst}$ (Xu and Zhou, 2008). We did not succeed in finding unequivocal relationships between $PWUE_{in}$ and stomatal traits in *B. pendula*.

3.2. Effects of exposure to increased air humidity on trees' gas exchange (II–IV)

The impact of elevated atmospheric humidity on trees' gas exchange is complex and depends on several other environmental factors. In summer of 2009 (**II**), we established differences only in basic photosynthetic parameters between saplings of silver birch growing in humidified (**H**) and control plots (**C**). The air humidity manipulation affected light-saturated net photosynthesis (A_{max}), maximum rate of Rubisco carboxylation ($V_{c\ max}$) and maximum rate of electron transport (J_{max}) – they all were lower in plants growing under increased RH. Regarding stomatal conductance and photosynthetic water-use efficiency, we got unexpected results: growing in higher RH affected neither g_S nor $PWUE_{in}$ (Table 1).

In 2009, the height and radial growth rates were lower in silver birch trees grown in higher RH (Table 1 in **II**) and hybrid aspen trees also had lower height increments when exposed to increased RH (Tullus *et al.*, 2012). Leuschner (2002) found that shoot nutrient concentrations were generally lower in herbaceous temperate woodlands species grown in higher RH.

Table 1. Comparison of gas exchange characteristics between the two treatments (N=6). R_d, dark respiration rate; ϕ, apparent quantum yield; θ, convexity of non-rectangular hyperbola; A_{max}, light-saturated rate of gross photosynthesis; Q_{comp}, light compensation point; Q_{sat}, light saturation point; g_S, stomatal conductance to water vapour measured at saturating irradiance; $PWUE_{in\ max}$, maximum intrinsic photosynthetic water-use efficiency; $PWUE_{in\ sat}$, intrinsic photosynthetic water-use efficiency measured at saturating light; c_i/c_a, ratio of intercellular to ambient CO_2 concentrations; $V_{c\ max}$, maximum rate of carboxylation; J_{max}, maximum rate of electron transport; V_{TPU}, triose phosphate utilisation rate; Γ, CO_2 compensation point; L_S, relative stomatal limitation to photosynthesis

Characteristic	Mean ± SE	
	Control	Humidification
R_d (μmol m^{-2} s^{-1})	–0.74±0.090	–0.88±0.081
ϕ (mol CO_2 mol $quanta^{-1}$)	0.064±0.0048	0.061±0.0022
θ	0.73±0.054	0.67±0.074
A_{max} (μmol m^{-2} s^{-1})	14.6±0.81	12.1±0.56*
Q_{comp} (μmol m^{-2} s^{-1})	11±1.6	15±1.9
Q_{sat} (μmol m^{-2} s^{-1})	254±15.2	235±10.8
g_S (mol m^{-2} s^{-1})	0.766±0.106	0.882±0.190
$PWUE_{in\ max}$ (μmol mol^{-1})	23.4±3.78	26.6±3.44
$PWUE_{in\ sat}$ (μmol mol^{-1})	21.4±2.51	22.3±5.88
c_i/c_a	0.77±0.014	0.80±0.021
$V_{c\ max}$ (μmol m^{-2} s^{-1})	71.2±6.97	56.8±2.23*
J_{max} (μmol m^{-2} s^{-1})	251±11.9	187±14.1*
V_{TPU} (μmol m^{-2} s^{-1})	24.4±4.00	19.3±3.10
Γ (μmol mol^{-1})	9.6±0.33	10.4±0.51
L_S (%)	30.3±4.60	25.7±2.68

Statistical significance for the difference between the treatments: $^*P<0.05$

However, total element uptake into the shoot increased with higher RH, thus the nutrients acquired from the soil were diluted by enhanced assimilation of carbon under high air humidity. In our study, plants grown in higher RH had lower foliar N and P concentrations, lower P:N ratio (Table 3 in **II**), and carbon assimilation and growth rates declined due to increased RH. Therefore, the leaf-level nutrient dilution is excluded. The foliar P:N ratio is considered even more important factor than tissue concentrations of either macronutrient in determining nutrient limitations, being related to several leaf and whole-tree attributes (Aerts and Chapin, 1999; Specht and Specht, 2010). Gislerød and

Mortensen (1990) have shown that Elatior begonia (*Begonia×hiemalis* Fotsch) grown at elevated RH has decreased concentrations of N, P, and K in leaves and stems, and transpiration of the plants is 56% lower in the high-RH plants. Mortensen and Gislerød (2000) found no differences in the nutrient concentrations (N, P, K) of rose leaves in the two RH treatments, but the concentrations of Ca and Mg in the flower were lower in roses grown at higher RH. They also found that water consumption per unit leaf area was 25% lower in high-RH grown roses. All this evidence suggests that nutrient uptake depends on water fluxes through the plants modified by atmospheric humidity.

Artificial misting in the FAHM area reduces the humidity gradient between leaves and atmosphere and that reduces stem sap flux density (Kupper *et al.*, 2011). Tullus *et al.* (2012) attribute the lower growth rate of hybrid aspen grown in higher RH in 2009 primarily to reduced N supply due to decreased transpiration-driven mass flow. Therefore, we assume that increased RH during leaf expansion of silver birch trees alters water consumption and this decreases leaf N and P contents. $V_{c\,max}$ and leaf N content per leaf area are positively correlated (Grassi *et al.*, 2005; van de Weg *et al.*, 2012); we found that J_{max} was associated with leaf P content. The reduction of phosphate uptake, a relatively immobile nutrient relying primarily on mycorrhizal associations (Smith *et al.*, 2003; Plassard and Dell, 2010), can be explained by changes in fine root morphology and ectomycorrhizal fungal communities (Parts *et al.*, 2013). We conclude that diminished nutritional status of leaves in humidity-treated trees reduces photosynthetic capacity (A_{max}, $V_{c\,max}$, J_{max}) in silver birch in moist summers (Fig. 2) without significant changes in stomatal regulation accompanied by growth retardation.

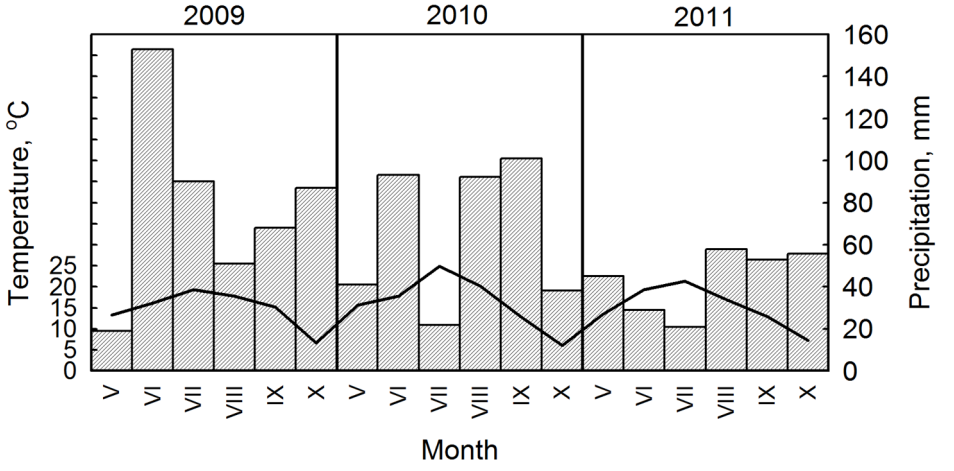

Figure 2. Weather data in the growing period of 2009 to 2011: the dark line indicates monthly average air temperature, and the grey bars indicate monthly precipitation (Figure designed by Arvo Tullus).

On saplings of hybrid aspen sampled in 2011 (**III**) we got results opposite to those obtained from silver birch: the humidification treatment influenced stomatal behaviour, but not photosynthetic parameters (Tables 2 and 3 in **III**). The air humidity treatment did not affect leaf photosynthetic capacity and, thus, we can exclude the humidity-driven effect on plant nutrient supply or damage of photosynthetic machinery. The absence of an impact of the humidity manipulation on photosynthetic machinery in hybrid aspen was confirmed by chlorophyll fluorescence measurements performed in the droughty summer of 2012. In contrast to photosynthetic parameters, the traits characterising stomatal conductance to water vapour and leaf WUE differed significantly between the humidified and control trees. Stomatal conductance depends strongly on soil water conditions: when soil water availability is low, plants control their water status by restraining transpiration via regulating g_S. During the gas exchange measurements, stomata in the control plants were more constrained (Fig. 3B) because soil water potential in **C** plots was significantly lower than in **H** plots (–194±4 and –138±4 kPa, respectively; $P<0.001$). Nevertheless, after including Ψ_S into analysis model, the treatment effect on g_S and $PWUE_{in}$ was still significant (Table 3 in **III**) attesting that differences between the treatments were caused not only by differences in soil water status.

In fact, the treatment effect on stomatal openness in the field experiment may reflect different acclimatory responses of stomata to various environmental factors during leaf development, high air humidity among other factors. As shown by Fanourakis *et al.* (2011) on rose (*Rosa hybrida* Vill.) plants, the degree of stomatal adaptation depends on both the timing and duration of exposure to high RH. In the FAHM experiment, increased RH decreases sap flux density in humidified trees (Kupper *et al.*, 2011; Tullus *et al.*, 2012); therefore the treatment effect represents primarily stomatal acclimation to modified atmospheric evaporative demand. At the same time, decreased AED and transpirational water loss from trees leave more water in the soil, thus there is higher soil water potential in **H** plots during and after the leaf expansion (Fig. 2 in **III**). Consequently, the treatment effect on stomatal behaviour is mediated at least partly by changes in soil water content (Fig. 3). Strict separation of plant responses between these two environmental factors in field experiments (i.e. under uncontrolled conditions) is impossible as they are connected to each other.

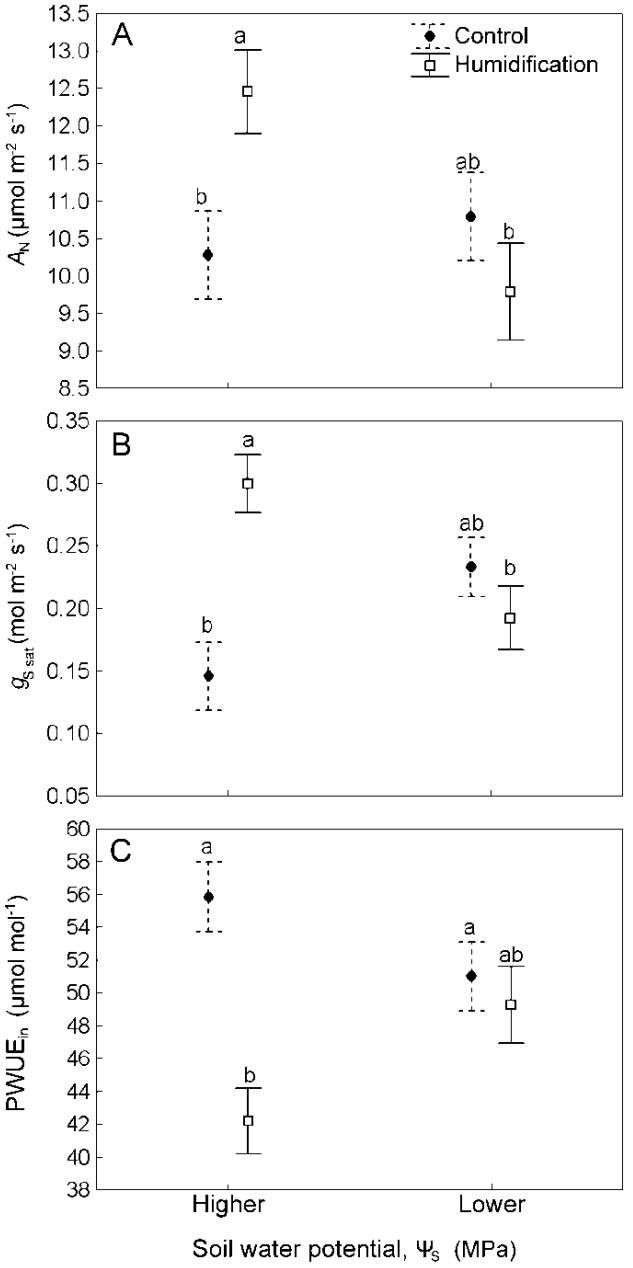

Figure 3. Means of net photosynthesis (A_N; **A**), stomatal conductance to water vapour at saturating Q ($g_{S\ sat}$; **B**) and initial intrinsic water-use efficiency (PWUE$_{in}$; **C**) in control (closed circles) and humidified trees (open squares) of hybrid aspen depending on soil water potential at a depth of 30 cm. Values are means±SE; different letters denote statistically significant (P<0.05) differences.

Direct comparison of the data obtained from hybrid aspen and silver birch is complicated. First, these are two different species; although both are fast-growing and light-demanding deciduous trees, expectation of similar response to rise in RH is not reasoned. Possen *et al.* (2011) showed that silver birch and European aspen (one of the parent species for hybrid aspen) have different strategies to cope with soil water shortages. Hence, the response to exposure to elevated RH may be species specific. Second, the study years differed in weather conditions. The summer of 2011 was very droughty, while the summer of 2009 was moderately rainy (Fig. 2). The summer of 2010 (**IV**) was also dry, but not as droughty as the summer of 2011. In 2010, there were no differences in A_N, g_S and $PWUE_{in}$ in *B. pendula* between the treatments, but leaf conductance to water vapour (i.e. total gaseous phase conductance; g_L) measured in ambient conditions was higher in **H** treatment than in the control (Table 2 in **IV**). It seems that silver birches growing in increased RH environment behave in dry summers similarly to hybrid aspen by maintaining higher stomatal openness, primarily due to greater soil water reserves left in **H** plots. Differences in acclimation of gas exchange to elevated air humidity may result not so much from species-specificity, but from differences between growth conditions while leaves are expanding.

Many works on different species have showed that plants grown in higher air humidity have higher growth rate and greater biomass compared to those grown at lower humidity (Gislerød and Mortensen, 1990; Hirai *et al.*, 2000; Leuschner, 2002; Codarin *et al.*, 2006; Lendzion and Leuschner, 2008; Ben-Asher *et al.*, 2013; Roriz *et al.*, 2014). Permanently decreased RH may threaten the vitality and survival of woodland herbs (Lendzion and Leuschner, 2009), which may change the distribution of some herbaceous species susceptible to RH level (Leuschner and Lendzion, 2009). Although hybrid aspen trees grown under increased atmospheric humidity demonstrated lower growth rates across the 6-year study period (Rosenvald *et al.*, 2014), relative height growth in 2011 was greater in high-RH grown trees (Table 4 in **III**). Reduced stomatal limitation of photosynthesis in trees grown at elevated air humidity (37.7 versus 41.3% in humidified and control plants, respectively) allowed slightly higher net photosynthesis (Table 2 in **III**) and bigger relative current-year height increments than in trees exposed to ambient air humidity. This indicates that growing in higher RH is beneficial to trees during moderate droughts, as these trees are able to keep stomata more open at the cost of lower $PWUE_{in}$. This response suggests a mitigating effect of higher air humidity on trees under mild water stress induced by lowering soil water availability. To summarise, the results of the experiment conducted on silver birch and hybrid aspen in field conditions suggests that the impact of increased RH depends significantly on other factors, including weather conditions prevailing during the growing season.

3.3. Rapid and long-term effects of limited water supply on leaf gas exchange (III, IV)

Rapidly-induced water deficit quantified by leaf (Ψ_L) or branch water potentials (Ψ_B) has a significant effect on gas exchange parameters in *B. pendula*. Stomatal closure is a universal plant response to rapidly imposed water stress, followed by a decrease in A_N. In silver birch we established a very tight relationship (R^2=0.970, P<0.001) between g_S and A_N (Fig. 3 in **IV**). At first $PWUE_{in}$ increased with decreasing g_S in response to falling water potential, at g_S ~0.06 mol m^{-2} s^{-1} it reached a maximum and thereafter declined very steeply as A_N decreased more rapidly than g_S. This happened at Ψ_B<−1.0 MPa, indicating a direct impact of water deficit on photosynthetic machinery. Similar patterns of g_S versus A_N were observed in hybrid aspen, in response to phloem girdling inducing stomatal closure, although Ψ_L even increased (Sellin *et al.*, 2013). The data analysis revealed that branch water potential was a more relevant factor than Ψ_L in governing responses of gas exchange parameters to water deficit (Table 1 in **IV**). Thus, the gas exchange and stomatal conductance of silver birch are determined by direct water availability to the leaf, estimated by Ψ_B in the petiole insertion point, rather than by the current leaf water status (Ψ_L) itself. The relationship between gas exchange and Ψ_B is probably mediated by stem hydraulic capacitance, and it supports the idea that stomatal openness is regulated to prevent stem rather than leaf xylem dysfunction in case of water deficit (Meinzer *et al.*, 2009). This is likely a general trait for broad-leaved trees, reported also for a number of subtropical tree species (Zhang *et al.*, 2013). Co-ordination of liquid and gaseous phase conductances in silver birch while adjusting to water deficit is confirmed by the strong positive relationship between A_N and g_S and plant hydraulic characteristics – soil-to-branch hydraulic conductance and whole-tree conductance (Fig. 5 in **IV**).

As the experiment was carried out at the FAHM site, we observed certain differences between the humidified and control plots. Under moderate water deficit (Ψ_L≥–1.55 MPa), leaf conductance to water vapour (g_L), transpiration rate and leaf hydraulic conductance (K_L) were higher (P<0.05) and leaf temperature lower in trees grown in elevated air humidity than in control trees. Under severe water deficit (Ψ_L<–1.55 MPa), the treatments showed no any difference. The humidification manipulation affected most of the studied characteristics, but not A_N and $PWUE_{in}$. In July, soil water potential underwent a substantial decline representing a long-term water deficit. After including Ψ_S into the analysis models, the effect of humidification treatment became insignificant for all gas exchange characteristics except one (g_L measured at ambient conditions; Table 4 in **IV**). Hence, the treatment effect was to a great extent realized through changes in soil water availability, i.e. due to higher soil water potential in **H** treatment. Two functional characteristics – g_L and K_L – exhibited higher (P<0.05) sensitivity to water deficit in trees grown under increased air humidity (Fig. 4). However, the birch trees showed differential changes in these

two fundamental traits due to the experimental manipulation: the humidity-treated trees exhibited substantially faster water deficit-driven reduction in K_L than in g_L when compared to the control. The disproportionate changes in sensitivity of stomatal versus leaf hydraulic conductance to water deficit will impose greater risk of desiccation-induced hydraulic dysfunction on the plants grown under high atmospheric humidity, in case of sudden weather fluctuations, and might represent a potential threat to hemiboreal forest ecosystems.

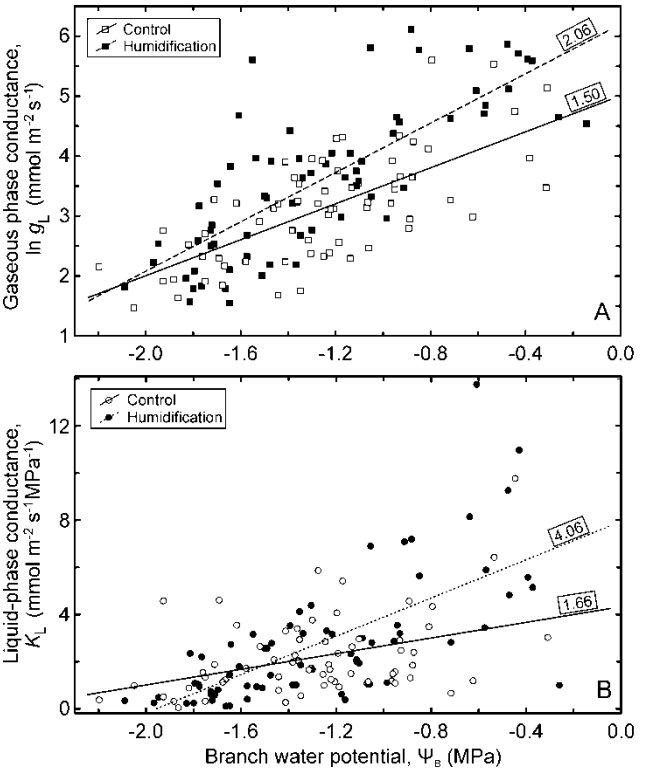

Figure 4. Branch water potential (Ψ_B) versus leaf conductance to water vapour (g_L; **A**) and leaf hydraulic conductance (K_L; **B**) in control and humidified trees of silver birch. The numbers by the regression lines indicate the respective slopes.

During evolution, plants have evolved a variety of structural traits and physiological mechanisms to avoid tissue dehydration, i.e. maintaining tissue water potential above critical threshold. Dehydration avoidance in different species is associated with various adaptive traits which minimise water loss and/or maximise water uptake (Chaves *et al.*, 2003). However, plant response to drought stress depends on how quickly it is imposed (Kim and van Iersel, 2011); therefore, one should distinguish long- and short-term responses to water stress. Long-term acclimation includes structural adjustments that maintain homeostasis

between water supply, water demand, and plant metabolism, whereas over short-term water stress plants try to maintain leaf hydration level by stomatal closure (Chaves *et al.*, 2003; McDowell *et al.*, 2008; Arve *et al.*, 2011). The two-stage data analysis proved that A_N and g_S in silver birch were affected by both, by rapid and long-term water deficit (quantified by branch and soil water potentials, respectively), although admittedly their effects are not strictly separable in a field experiment.

Based on the results, one could conclude that long-term water stress did not affect $PWUE_{in}$ in silver birch, whereas short-term stress did (Tables 1 and 4 in **IV**). Thus, the observed stomatal responses were associated primarily with the impact of rapidly-induced water deficit and obviously driven by hydraulic signals, because $dg_L/d\Psi_B$ did not differ between the treatments in intact branches and did not depend on soil water status. Some researchers (Aasamaa and Sõber, 2011; Brodribb and McAdam, 2011) highlight the priority of hydraulic versus metabolic stimuli: it is considered fundamentally important in preventing plant desiccation and is maintained in stomatal control through vascular plant phylogeny. Evidently the decline in Ψ_S was not so large (Fig. 4 in **IV**) to affect $PWUE_{in}$, while direct availability of water to the leaves (Ψ_B) fell sharply by the factor of ~2.5 on a short time scale. Silver birch has been reported to be able to acclimate efficiently to lack of water, including adjustment of WUE (Possen *et al.*, 2011). Consequently, the drought developed in Estonia in summer 2010 was not severe enough to induce significant changes in photosynthetic water-use efficiency.

In summer months of 2011, there was less rainfall in Estonia (Fig. 2) and plants suffered more intense drought than in 2010. Daily average canopy conductance to water vapour (g_C) also decreased with declining Ψ_S ($P<0.001$), while the treatments demonstrated contrasting sensitivities ($dg_C/d\Psi_S$) to developing soil water deficit: the corresponding slopes were 0.94 and 3.01 for control and humidified trees, respectively (Fig. 5). As stomatal sensitivity to atmospheric evaporative demand was unaffected by the experimental manipulation (Fig. 5A in **III**) and the plants in both treatments experienced comparable ranges of soil water potential, the differential response of g_C to decreasing soil water availability was probably mediated by plant hydraulic conductance (Cohen and Naor, 2002; Domec *et al.*, 2009). Hydraulic measurements performed on intact aspen trees in 2010 revealed that trees' hydraulic conductance expressed per unit leaf area was smaller in **H** trees (A. Sellin, unpubl. data). Under conditions of soil water deficit, the lower hydraulic capacity probably becomes a crucial factor for high-RH grown trees, limiting leaf water supply and inducing a steep decline in canopy conductance.

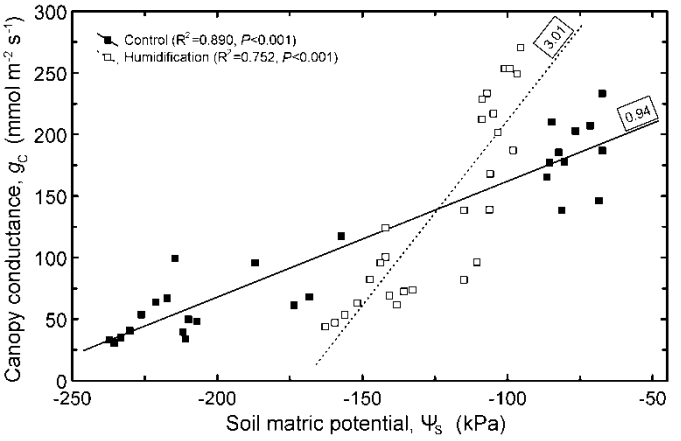

Figure 5. Variation in daily average canopy conductance to water vapour (g_C) in control and humidified trees of hybrid aspen depending on bulk soil water potential (Ψ_S). The numbers by the regression lines indicate the respective slopes.

3.4. Stomatal responses to fast decline in air humidity (III, V)

The experiments performed in both silver birch and hybrid aspen at the FAHM site suggest that air humidity manipulation affects stomatal sensitivity to the factors causing their closure. In 2013 (**V**) we conducted an experiment to assess stomatal sensitivity to fast decreasing RH/increasing VPD as a closure-inducing factor. Hybrid aspen coppice grown in humidified plots has significantly more sensitive stomata to increasing leaf-to-air vapour pressure difference (VPD_L) compared to control plants. This applies both across the entire data set (P<0.001; Fig. 6A) and also when analysing only the closing response (P<0.001; Table 2 in **V**). Li and Li (2014) compared two species inhabiting different climate zones and found that trees grown in climates with higher RH have more sensitive stomata compared to trees grown in lower RH zones. There was also higher leaf ABA concentration in trees grown in higher RH. Ψ_S in our study did not differ between the treatments during the study period, thus stomatal sensitivity was not affected by root-borne ABA levels.

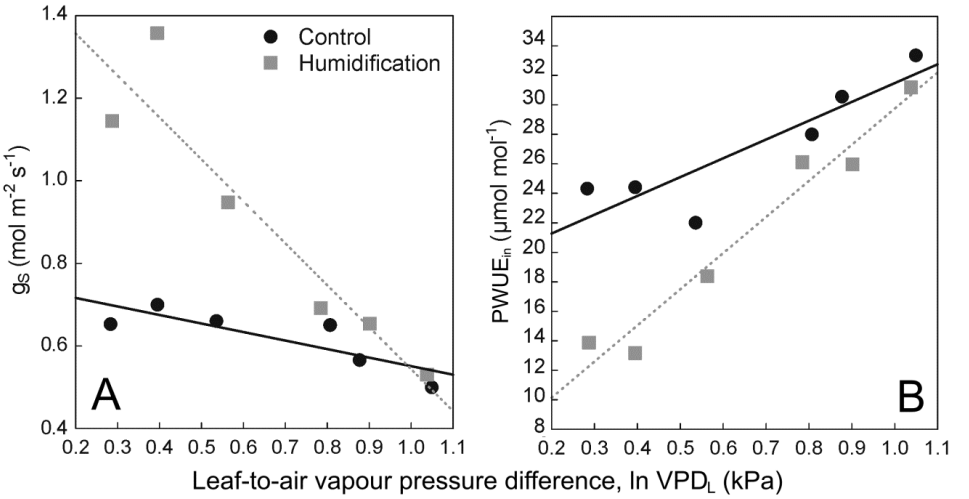

Figure 6. Relationships between leaf-to-air vapour pressure difference (VPD_L) and stomatal conductance (g_S; **A**) and intrinsic water-use efficiency ($PWUE_{in}$; **B**) in coppice of hybrid aspen in control and humidification treatments. The points represent means of six data subsets per treatment.

Experiments indicate that actual ABA concentration itself is not a reason for low stomatal sensitivity, but a long period of low ABA will result in ABA desensitization accompanied by a decline in stomatal responsiveness (Aliniaeifard and Van Meeteren, 2013). It has been shown that growing in high RH decreases ABA level in plants (Rezaei Nejad and Van Meeteren, 2008; Okamoto *et al.*, 2009; Arve *et al.*, 2013; Aliniaeifard *et al.*, 2014), explainable by increased activity of ABA 8'-hydroxylase both in stomata and vasculature (Okamoto *et al.*, 2009). The occurrence of ABA-mediated effect should lead to lower stomatal sensitivity in humidity-treated trees. Thus, our results differ from expectations and contradict other studies of various stomata closing factors (Torre *et al.*, 2003; Rezaei Nejad and Van Meeteren, 2005; Fanourakis *et al.*, 2011; Fanourakis *et al.*, 2013; Giday *et al.*, 2013; Aliniaeifard *et al.*, 2014; Aliniaeifard and van Meeteren, 2014; Arve *et al.*, 2014). The abovementioned works have been conducted on plants growing in greenhouses or growth chambers in stable environments applying big shifts in RH. Our study and that of Li and Li (2014) were conducted in nature, in a daily and seasonally variable environment – under uncontrolled conditions. Daily average RH across the growing period in our study was elevated by only 3–4%, while for example a difference in RH of 35% was applied in the experiment by Aliniaeifard *et al.* (2014). The plants grown under increased RH demonstrated both lower stomatal density and stomatal pore area index, whereas the length of stomatal pore was unaffected by the manipulation. We found no correlations between stomatal morphological indices and functional parameters in hybrid aspen, suggesting that stomatal sensitivity was unaffected by stomatal morphology.

The consequences of enhanced stomatal sensitivity induced by increased air humidity on plant performance are unclear. This may be beneficial to plants in daily timespan and is considered to be associated with growing in natural environments, reflecting leaf acclimation to dense canopies (Talbott *et al.*, 2003). Under dense, sheltered canopies, leaves experience poor light conditions, lowered ambient CO_2 concentration, and elevated RH. Under these conditions, light-stimulated stomatal opening is reduced, possibly limiting photosynthesis through inadequate CO_2 uptake, while high RH acts as a signal enhancing stomatal sensitivity and accelerating their opening. Thus, sensitive regulation of g_S and $PWUE_{in}$ may provide benefits for tree seedlings growing in the forest understory characterised by high RH and permanently changing sunfleck patterns, thereby helping to compete and survive in this highly dynamic environment.

The increased sensitivity of g_S observed in aspen coppice grown in artificially elevated RH causes also higher sensitivity of $PWUE_{in}$ to fast decrease in VPD_L (Fig. 6B). Comparing the slopes of stomatal sensitivity to VPD_L (m) relative to reference stomatal conductance at VPD_L= 1kPa (g_{Sr}) of both treatments with theoretical slopes by Oren *et al.* (1999) suggests that **H** trees are characterised by strict stomatal control over leaf water loss, whereas the respective slope of **C** trees is more similar to drought-tolerant species with less strict regulation of Ψ_L. As high $PWUE_{in}$ and its fast adjustment via regulation of g_S are considered beneficial at low water availability, growing in higher RH may improve tree performance in fluctuating environment. In light of the rising atmospheric humidity predicted for high latitudes, strict stomatal control over water loss might be beneficial for trees if drought events become more frequent in the future. Although increased stomatal sensitivity provides more efficient control over water loss in case of mild droughts, further increases in atmospheric RH at high latitudes is probably not beneficial to hybrid aspen in the long term (Tullus *et al.*, 2012).

Analysing g_S response to rising VPD_L on intact plants, we detected 23 response curves, where g_S decreased as VPD_L increased (decreasing response, DR) and 51 curves where g_S increased with rising VPD_L (increasing response, IR; Fig. 3 in **V**). IR response occurred at higher frequency (55% of all cases) in control plants. Plants that demonstrated IR response were exposed to harsher conditions before gas exchange measurements: air temperature and photosynthetic photon flux density were higher, while RH was lower than before DR response (Table 1 in **V**). This finding implies that water conservation via strict stomatal control was not the prevailing behaviour for the plants when exposed to fast-increasing AED. Thus, about two-thirds of all experimental shoots showed stomatal opening as VPD_L increased. The substantially harsher conditions experienced by these plants prior to gas exchange measurements resulted in lower Ψ_L and almost closed stomata (Table 1 in **V**), which opened in response to increasing VPD_L.

Zhou *et al.* (2010) demonstrated on *Populus euphratica* trees that in case of heat shock and mild water deficit combined, trees keep some stomata open at a cost of lower $PWUE_{inst}$ to accelerate transpiration and to dissipate overheating and protect photosynthetic machinery against high-temperature damage. Stomata of *Vitis vinifera* L. stay relatively open when Ψ_L decreases with increasing VPD while growing in ample soil moisture conditions (Rogiers *et al.*, 2012). It has been shown that well-irrigated *Triticum aestivum* L. plants open their stomata in response to declining air humidity; they have also higher hydraulic conductivity in drier air (Kudoyarova *et al.*, 2007). Opening of stomata in response to decreasing air humidity probably promotes rapid growth and higher productivity under well watered conditions, whereas high hydraulic conductivity permits easy replacement of water lost through transpiration with water from soil (Kudoyarova *et al.*, 2007). The aspen plants grew in moist soil conditions: the average soil water potential was –19 kPa in **C** plots and –26 kPa in **H** plots during the study period in 2013; in the dry summer of 2011 Ψ_S values varied from –50 to –250 kPa through three summer months (Figure 2 in **III**). In addition to high soil water availability, our experiment was conducted on 4–5 month old coppice shoots; the plants used the large root systems of 8-year-old trees harvested the previous autumn.

The young age of plants is also a relevant factor of stomatal responses. Fast-growing seedlings tend to maximize productivity by maintaining high g_S under favourable conditions until the roots of the plants develop access to deeper water reserves or the canopy closes, and thus have lower WUE despite of the high risk of desiccation-induced mortality (Cavender-Bares and Bazzaz, 2000; Juarez-Lopez *et al.*, 2008; Forrester *et al.*, 2010; Su *et al.*, 2014). The aspen plants probably opened their stomata with rising VPD_L to prevent heat damage to photosynthetic apparatus (also g_S was very sensitive to higher temperatures as shown in Fig. 4a in **V**), to provide sufficient foliage cooling and to maintain higher productivity without the need to conserve water, because there was ample soil moisture and water uptake from the soil relied on large root systems.

Opening of stomata at the expense of water loss in response to normal stomatal closure-inducing environmental changes is probably the strategy of young trees for fast establishment, especially under conditions of high irradiance or VPD. Growing in higher relative humidity may enhance stomatal sensitivity and therefore may improve water use during fast changes in the environment. Aasamaa and Sõber (2011) suggest that weak stomatal control over water loss is beneficial to fast-growing trees to achieve higher carbon gain in changing environments.

We saw that air humidity manipulation influences also stomatal responses to CO_2 concentration in hybrid aspen saplings (**III**). g_S decreased with increasing external CO_2 concentration (c_a), but this response did not depend on the treatment (humidified versus control plots). By contrast, A_N increased significantly ($P<0.001$) faster and $PWUE_{in}$ significantly ($P<0.001$; Fig. 7 in **III**) slower with increasing c_a in trees grown in elevated RH compared to control trees. Unlike

the $A_N=f(c_a)$ slopes, we did not detect differences in $A_N=f(c_i)$ slopes between the treatments. The differential response of A_N to external versus internal [CO_2] (c_i) once more confirms that photosynthetic machinery of hybrid aspen was not affected by growing in elevated atmospheric humidity; the differences in gas exchange parameters result rather from stomatal acclimation to different humidity levels. Therefore, $PWUE_{in}$ turns out to be less sensitive to [CO_2] in trees grown in higher RH.

For several species, growing in higher RH reduces stomatal closure in response to closing stimuli like desiccation, increasing [ABA] and dark periods (Torre *et al.*, 2003; Rezaei Nejad and Van Meeteren, 2005; Fanourakis *et al.*, 2011; Fanourakis *et al.*, 2013; Aliniaeifard *et al.*, 2014; Aliniaeifard and van Meeteren, 2014; Arve *et al.*, 2014). In our study (**III**), the responses of $PWUE_{in}$ imply that also hybrid aspens grown in higher relative humidity should have less sensitive stomata. Of course, the lower slope of $PWUE_{in}=f(c_a)$ established in humidity-treated trees does not necessarily mean that leaves from different treatments have different acclimations with respect to air humidity. As Ψ_S was higher in humidified plots, stomata in these trees had to control water losses not as strictly as in control trees (Fig. 6B in **III**), which could result in a lower response of $PWUE_{in}$ to [CO_2] in **H** trees. Nevertheless, one cannot exclude an acclimatory response directly to elevated air humidity.

4. CONCLUSIONS

The following conclusions can be drawn based on this thesis:

1. Leaf water-use efficiency (WUE) in crowns of *B. pendula* is characterised by considerable spatial and temporal plasticity, contributing to efficient utilization of the microenvironment existing within a forest canopy. Under unlimited light conditions, the lower-canopy leaves of the trees growing in forest stands have inherently higher photosynthetic WUE compared to the upper canopy because of hydraulic constraints implying potentially greater limitations of water supply to lower-canopy foliage. Low WUE of intact shade foliage is environmentally induced, ensuing primarily from the light limitation of photosynthesis in lower canopy layers. The inverse height-related trends in intrinsic WUE and hydraulic conductance within crowns of trees at sufficient light intensities support our first hypothesis, referring to the trade-off between water transport and use efficiencies.
2. The impact of long-term exposure to increased air relative humidity (RH) on trees' gas exchange is complex and depends on other environmental conditions. In a wet year, the trees acclimated to higher RH exhibited decreased photosynthetic capacity because of diminished nutrient uptake from soil due to smaller transpirational water fluxes through the trees. Stomatal conductance to water vapour and intrinsic WUE were not affected by air humidity manipulation. In a dry year, trees behaved *vice versa* – basic photosynthetic parameters remained unaffected, while increased atmospheric humidity enhanced stomatal conductance accompanied by decreased WUE. Hence, the second hypothesis (trees growing under increased RH have leaves with lower phtotosynthetic WUE) is valid only in dry years. Increased air humidity mitigates effects of mild water stress on trees by lowering stomatal limitation of photosynthesis and allowing a high growth rate in fast-growing tree species.
3. Broadleaved trees acclimated to higher air humidity develop leaves more sensitive to rapidly-induced water deficit both in terms of leaf conductance to water vapour (g_L) and leaf hydraulic conductance (K_L). Our results do not support the third hypothesis that trees exposed to humid air have lower sensitivity to stomatal closure-inducing factors (i.e. water deficit). A rapidly-induced water deficit overrode the effect of long-term water stress due to seasonal soil drying on photosynthetic WUE. Trees grown under conditions of elevated RH exhibit a faster water deficit-driven reduction in K_L relative to g_L. The disproportionate changes in sensitivity of stomatal versus leaf hydraulic conductance to water deficit will impose greater risk of desiccation-induced hydraulic dysfunction on plants developed under high atmospheric humidity during sudden weather fluctuations, and might represent a potential threat to hemiboreal forest ecosystems.
4. Stomata of leaves developed under increased RH are more sensitive to fast decreases in air humidity, refuting the third hypothesis (expected lower sen-

sitivity to stomatal closure-inducing factors – increasing atmospheric vapour pressure deficit). In the context of the long-term increasing atmospheric humidity predicted for Northern Europe, enhanced stomatal sensitivity might be beneficial for trees by allowing quick adjustment of gas exchange and promoting their acclimation to weather extremes (severe drought, heat wave) becoming more frequent in the future. However, effective control over transpirational water losses via strict stomatal regulation is not the main strategy of young fast-growing trees (like coppice shoots), but they may open their stomata with rising VPD to prevent heat damage and maintain a positive carbon balance with the greater risk of tissue dehydration.

5. The expected climate change-induced increase in the growth rate of trees at northern latitudes (in boreal and hemiboreal forest zones) resulting from the earlier start of the growing season in spring (Jarvis and Linder, 2000) and higher carbon assimilation rate due to warming and rising [CO_2] (Way and Oren, 2010; Mäenpää *et al.*, 2011) might be counteracted by other physiological mechanisms (diminished nutrient uptake, reduced photosynthetic capacity, stomatal limitation caused by decreased hydraulic conductance, higher risk of hydraulic dysfunction, etc.) if the temperature rise is accompanied by an increase in rainfall frequency and atmospheric relative humidity.

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SUMMARY IN ESTONIAN

Keskkonnafaktorite mõju heitlehiste puude gaasivahetusele: fookuses fotosünteesi veekasutuse efektiivsus

Fotosünteesi – protsessi, mille käigus valgusenergia muundatakse keemiliseks energiaks – tähtsust biosfääri toimimises on võimatu alahinnata. Protsess on algselt välja kujunenud veelises keskkonnas ja vajab toimumiseks stabiilset vee keskkonda, mis muudab selle vältimatuks ressursiks kõigi fotosünteesivate organismide jaoks. Esimeste taimede, kes tõenäoliselt mageveekogudest maismaale elama asusid, sisekeskkond sõltus vahetult nende väliskeskkonna veestaatusest. Kuna õhukeskkond põhjustab taimerakkude dehüdratatsiooni ning see kahjustab fotosünteesiaparaati ja piirab nende kasvu, suutsid esimesed taimed levida vaid väga niisketes kasvukohtades. Taimeriigi pika evolutsiooni käigus kujunesid välja sellised liigid, kes suudavad säilitada fotosünteesiks vajalikku rakkude hüdratatsiooni taset tänu erinevate vee auramist vähendavate struktuuride (kattekoed, kutiikula) kujunemisele taimekeha välispinnal. Need kaitsvad kihid on aga ühtaegu raskesti läbitavad nii veeaurule kui ka süsinikdioksiidile (CO_2). Tagamaks fotosünteesi varustamise vajaliku substraadiga (CO_2), kujunesid aja jooksul taimede epidermis õhulõhed. Kuna keskkonnatingimused on ajas varieeruvad, siis selleks, et neid kahte vastassuunalist gaasivoogu kontrollida, arenesid õhulõhede ümber sulgrakud, mille turgori muutmise teel saab taim oma veekadu ja CO_2 omastamist reguleerida. Seega, mida kõrgem on fotosünteesi tase kaotatud veekoguse kohta (fotosünteesi veekasutuse efektiivsus), seda ökonoomsemalt taim veevarusid kasutab.

Kõrge veekasutuse efektiivsus (laiemas tähenduses) on taimede ellujäämise üks võtmefaktoreid keskkonnas, kus vett on vähe või kus sademed jaotuvad sesoonselt väga ebaühtlaselt. Suur veekasutuse efektiivsus on iseloomulik ariidsetel aladel kasvavatele taimedele. Samas ei ole kõrge veekasutuse efektiivsus kasulik üksnes väikese mullavee sisaldusega keskkondades, sest puittaimedel võib esineda veepuudusest tingitud stress ka nende suurte mõõtmete tõttu (hüdraulilise limitatsiooni hüpotees). Puude suur kõrgus pikendab vee transpordi teekonda mullast lehtedesse – suurema gravitatsioonipotentsiaali ja pikema hõõrdumisteekonna tõttu on kõrgete puude ladvas lehtede veevarustatus limiteeritum ja seega peaksid suurte puude lehed vett efektiivsemalt kasutama. On näidatud, et mulla veesisalduse vähenemise korral toimub lehe veekasutuse efektiivsuse regulatsioon erinevate valgustingimustega kohanenud taimedes erinevalt. Metsas, puistu sees erineb puuvõra alaosa keskkond ka sellest, mis on ladvaosas: võrastiku alumises osas on väiksem tuulekiirus, kehvemad valgustingimused ning niiskem õhk. Eelnevates töödes on leitud, et looduslikus puistus kasvava arukase (*Betula pendula* Roth.) võra alaosas kasvavad lehed on hüdrauliliselt rohkem limiteeritud võrreldes ülaosa lehtedega. Seega peaksid alumised, veevarustuselt potentsiaalselt tugevamini limiteeritud lehed olema efektiivsemad vee kasutajad. Selle kontrollimiseks viidi läbi lehtede gaasi-

vahetuse mõõtmised looduslikus segametsas, arukase võras, kus lehestik on kohanenud puistus esinevate vertikaalsete keskkonnagradientidega.

Valitsustevaheline Kliimamuutuste Nõukogu (ingl. *IPCC*) ennustab, et käesoleva sajandi jooksul kasvavad atmosfääri CO_2 kontsentratsioon, maakera keskmine temperatuur ning ekstreemsed ilmastikuolud sagenevad. Samas, erinevalt Lõuna- ja Kesk-Euroopast ennustatakse Põhja-Euroopale suuremat vihmasadude sagedust. Sagedasemad vihmahood koos tõusva temperatuuriga põhjustavad tulevikus Põhja-Euroopas atmosfääri suhtelise õhuniiskuse suurenemist. Seda, kuidas suurenev õhuniiskus mõjutab looduses kasvavate kiirekasvuliste ja valgusnõudlike lehtpuuliikide gaasivahetust, nende toimetulekut järskude keskkonnafluktuatsioonidega (ootamatu põud, kuumalaine), ei ole varem uuritud ja seega on õhu suhtelise niiskusesisalduse kasvu tagajärjed metsaökosüsteemidele teadmata. Maailma mastaabis unikaalne Metsaökosüsteemi Õhuniiskusega Manipuleerimise Eksperiment (ingl. *FAHM*), mille raames enamus antud teesides esitatud tulemusi on saadud, võimaldab meil seda lünka täita. Eksperimendid laboritaimedega on näidanud, et suurendatud õhu niiskusesisaldus mõjutab eelkõige õhulõhede tundlikkust nende sulgumist esilekutsuvate keskkonnafaktorite suhtes. Seega kontrollitakse antud töös, kas kõrgema atmosfääri niiskusetasemega kohanemine muudab ka looduses kasvavate taimede õhulõhede reaktsioone – tundlikkust mulla veedefitsiidi ja veeaururõhu kiirete muutuste suhtes.

Töö eesmärgid olid:

- Uurida gaasivahetuse vertikaalset ja päevast varieeruvust puistus kasvava heitlehise puu võras, keskendudes fotosünteesi veekasutuse efektiivsusele.
- Hinnata kunstlikult suurendatud suhtelise õhuniiskuse mõju lehe gaasivahetuse põhilistele parameetritele laialehistel puudel.
- Teha kindlaks, kuidas mõjutab kiire ja pikaajaliselt kujunev veedefitsiit erinevates õhuniiskuse tingimustes kasvanud puude gaasivahetust.
- Teha kindlaks, kuidas erineb kõrgema õhuniiskusega kohanenud lehtede õhulõhede käitumine tavatingimustes kasvanud lehtedest, kui veeaururõhkude erinevus lehe intertsellulaaride ja atmosfääri vahel suureneb.

Doktoritöös kontrolliti järgmisi hüpoteese:

1. Kuna võra alaosas, varjutingimustes kasvavad lehed on hüdrauliliselt tugevamini limiteeritud võrreldes võra ülaosas kasvavate hästivalgustatud lehtedega, siis on alumistele lehtedele iseloomulik kõrgem sisemine fotosünteesi veekasutuse efektiivsus (ingl. *intrinsic water-use efficiency*).
2. Kõrgema õhuniiskusega keskkonnas kasvavate puude lehed on madalama fotosünteesi veekasutuse efektiivsusega.
3. Kõrgema suhtelise õhuniiskusega aklimatiseerunud puude õhulõhed on vähem tundlikud õhulõhesid sulgevate faktorite (limiteeritud veevarustus; veeaururõhkude erinevuse suurenemine lehe intertsellulaaride ja atmosfääri vahel) suhtes.

Mõõtmised looduslikus puistus *in situ* näitasid, et arukase ülemiste lehtede veekasutuse efektiivsus on kõrgem kui alumistel lehtedel. Selles tulemuses ei ole midagi uut, sest ülemised lehed on paremini valgustatud ja seega nende netofotosünteesi tase ka kõrgem. Kuna netofotosünteesi intensiivsus ja õhulõhede veeauru juhtivus on omavahel seotud, on ka ülemiste lehtede õhulõhede juhtivus suurem. Samas kinnitasid tulemused selgelt, et juhul kui mõlemast võrakihist pärit lehed paiknevad piisava valgustatuse (Q>800 μmol m^{-2} s^{-1}) tingimustes, on hoopis alumiste lehtede veekasutuse efektiivsus kõrgem võrreldes ülemiste lehtedega. Enamgi veel, laborikatsed metsapuudelt lõigatud võrsetega (s.t. enamus hüdraulilisest takistusest on kõrvaldatud) kinnitasid veel kord, et alumise võrakihi lehed on seesmiselt efektiivsemad vee kasutajad.

Saadud tulemustest järeldub, et lehed on kohanenud vastavale võrakihile omaste valgustingimuste ja võrasiseste hüdrauliliste piirangutega. Varjulehed on ilmselt kohanenud valguslaikude efektiivseks ära kasutamiseks võra alaosas. Olukorras, kus päikesevalgust on vähe ja netofotosüntees madal, on õhulõhed vähem avatud säästmaks vett puu ülaosa, fotosünteetiliselt aktiivse võraosa jaoks. Valguslaigu esinemise korral netofotosünteesi intensiivsus kiiresti kasvab, kuid õhulõhede juhtivus hüdrauliliste piirangute tõttu mitte niivõrd. Kõrgem veekasutuse efektiivsus aitab piirata kavitatsiooni arenemist valguslaiku sattunud võrsetes.

Vastassuunalised trendid lehe veekasutuse efektiivsuse ja hüdraulilise juhtivuse vahel arukase võras toetavad esimest hüpoteesi ning viitavad lõivsuhtele taime veekasutuse ja -transpordivõime vahel. Antud doktoritöö tulemused ei ole vastuolus hüdraulilise limitatsiooni hüpoteesiga, pigem täiendavad seda. Käesoleva töö aluseks olevad mõõtmised on tehtud sama kõrgusega puudel, mistõttu suurusest tulenevad efektid saame välistada. Gaasivahetuse hüdrauliline limitatsioon ei ilmne mitte üksnes seoses puu mõõtmete kasvuga, vaid ka ühe puu piires, võra sees.

Pikaajaline kõrgema suhtelise õhuniiskuse mõju lehtpuude gaasivahetusele sõltub olulisel määral teiste samal ajal puule toimivate keskkonnafaktorite mõjust. Niiskel aastal (sademeid kasvuperioodil üle keskmise) mõjutab puude eksponeerimine suuremale suhtelisele õhuniiskusele peamiselt fotosünteesi parameetreid. Kõrgema õhuniiskusega keskkonnas kasvanud puude fotosünteesivõime oli madalam kui kontrollringide puudel, sest töötlusest tulenev madalam atmosfäärinõudlus (ingl. *atmospheric evaporative demand*) aeglustab veevoogu läbi puude ja väheneb toitainete omastamine mullast väiksema massivoolu tõttu. Kuival suvel aga ei mõjutanud eksponeerimine kõrgemale õhu niiskusetasemele lehtede fotosünteesi, vaid õhulõhede juhtivust ja lehtede veekasutuse efektiivsust. Seega käesolevas doktoritöös püstitatud teine hüpotees (kõrgemas suhtelises õhuniiskuses arenenud lehed on madalama fotosünteesi veekasutuse efektiivsusega) leidis kinnitust vaid kuival aastal. Gaasivahetuse ja puude juurdekasvu andmeid kõrvutades ilmneb, et atmosfääri suurenenud suhteline niiskusesisaldus vähendab kuival suvel fotosünteesi stomataarset limitatsiooni ja leevendab mõõduka põua negatiivset mõju puude juurdekasvule.

Eksperiment arukase lõigatud okstega, mida lasti vabas õhus kuivada tekitamaks lehtedes kiiret veedefitsiiti mõõtes samal ajal gaasivahetust, näitas, et kõrgema õhuniiskusega kohanenud lehtede õhulõhed reageerivad tundlikumalt oksa veepotentsiaali kiirele langusele võrreldes kontrollringide puudega. Seega kolmas püstitatud hüpotees ei leidnud kinnitust. Sama katse näitas, et ka lehe hüdrauliline juhtivus väheneb koos oksa veepotentsiaal langusega ning see langus on oluliselt järsem niiskemas õhus kasvanud puudel. Iseenesest ei ole kirjeldatud õhulõhede ja hüdraulilise juhtivuse reaktsioonides veedefitsiidile midagi uudset. Saadud tulemuste analüüs tõi aga välja selle, et suurenenud õhuniiskus muudab lehe vedela ja gaasilise faasi juhtivusi veepotentsiaali kiirete muutuste suhtes ebaproportsionaalselt: kõrgema õhuniiskusega kohanenud puude lehtede hüdrauliline juhtivus väheneb veepotentsiaali languse korral oluliselt kiiremini kui õhulõhede juhtivus. Lehe vedela ja gaasilise faasi juhtivuse tundlikkuse ebaproportsionaalne muutumine suurendab ekstreemsete ilmastikutingimuste korral hüdraulilise düsfunktsiooni riski niiskes õhus kasvanud puudes, mis võib tulevikus kujutada potentsiaalset ohtu hemiboreaalsetele metsaökosüsteemidele.

Arvukad laborikatsed erineva õhuniiskuse tingimustes kasvatatud taimedega on näidanud, et kõrgema suhtelise õhuniiskuse tingimustes arenenud lehtede õhulõhed on vähem tundlikud õhulõhede sulgumist indutseerivate keskkonnafaktorite suhtes. Käesolevas doktoritöös kontrolliti, kas ka mõneprotsendiline suhtelise õhuniiskuse suurenemine välitingimustes mõjutab õhulõhede reaktsioone kiiresti muutuva veeaururõhu suhtes. Katsed hübriidhaava (*Populus tremula* L. × *P. tremuloides* Michx.) kännuvõsudega näitasid, et kõrgemas õhuniiskuses kasvanud puude õhulõhed reageerivad hoopiski tundlikumalt atmosfäärinõudluse kiirele tõusule, lükates ümber doktoritöös püstitatud kolmanda hüpoteesi.

Põhja-Euroopale pikemas perspektiivis ennustatava suhtelise õhuniiskuse tõusu kontekstis on suurenenud õhulõhede tundlikkus metsapuudele kasulik, võimaldades ilmastiku ekstreemumite (põud, kuumalaine) ajal gaasivahetuse (s.h. fotosünteesi veekasutuse efektiivsuse) kiiret kohandamist vastavalt hetkeoludele. Samas ei pruugi tugev stomataarne kontroll veekadude üle olla parim strateegia tagamaks intensiivset kasvu ja kasvukohtade efektiivset hõivamist noorte taimede poolt, eriti kiirekasvulistel pioneerliikidel. Hübriidhaava kännuvõsudega läbiviidud eksperiment näitas, et umbes $^2/_3$ taimedest avas oma õhulõhed reaktsioonina veeaururõhkuse erinevuse tõusule intertsellulaaride ja väliskeskkonna vahel. Õhulõhede avamisega väldivad taimed kõrge temperatuuri kahjustavat mõju fotosünteesi aparaadile (intensiivsema transpiratsiooniga saavutatakse suurem lehte jahutav efekt), kuid see toimub taimekudede suureneva dehüdratatsiooni riski hinnaga. Selline oportunistlik strateegia on tõenäoliselt omane just noortele kiirekasvulistele ja valgusnõudlikele puittaimedele.

Atmosfääri suureneva CO_2 sisalduse ja keskmise temperatuuri tõusu põhjal prognoositud boreaalsete ja hemiboreaalsete alade metsapuude juurdekasvu intensiivistumine võib jääda väiksemaks või olla hoopis olematu, kui mainitud

kliimamuutustega kaasnevad sagedasemad vihmasajud ja suurenev atmosfääri suhteline niiskusesisaldus. Nimetatud kliimatrendid võivad kaasa tuua negatiivseid mõjusid puudele läbi erinevate protsesside – vähenenud toitainete omastamine mullast, fotosünteesivõime langus, hüdraulilise juhtivuse vähenemisest põhjustatud fotosünteesi stomataarne limitatsioon ning kõrgem hüdraulilise düsfunktsiooni risk.

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PUBLICATIONS

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